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THE RELATIVE ROLES OF TUMOR NECROSIS FACTOR ALPHA (TNF-A) AND INTERLEUKIN-1 (IL-1) IN THE INDUCTION OF INTERCELLULAR ADHESION MOLECULE-1 (ICAM-1) IN HUMAN EPIDERMIS BY ULTRAVIOLET RADIATION
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Low constitutive levels of expression of the adhesion molecule ICAM-1 (CD54) on the surface of keratinocytes within the epidermis is felt to be protective against cytotoxic damage by activated leukocytes. Induction of keratinocyte ICAM-1 is an early event in inflammatory skin diseases, correlated with keratinocyte activation and cytotoxicity.

Ultraviolet radiation (UVR) is an important inducer of ICAM-1 expression in the epidermis. In an extensive study of 21 different strains of human keratinocytes derived from different donors, we found that the level of induction of keratinocyte ICAM-1 expression in individual cell strains was similar with UVR and TNF-a (R=.591 p=.0047). Donors could be characterized as high responders or low responders to both TNF-a and UVR. Donors who were high responders to TNF-a in vitro also showed significant induction of keratinocyte ICAM-1 expression in the epidermis after UVR radiation in vivo. Low responders did not upregulate ICAM-1 in vivo.

Conversely, in cultured human neonatal and adult keratinocytes, IL-1 did not induce significant ICAM-1 expression. These cells contain large amounts of intracellular IL-1ra (icIL-1ra), the receptor antagonist for IL-1. In three different transformed human keratinocyte cell lines, the level of ICAM-1 induction by IL-1 varied in inverse relation to the level of icIL-1ra measured by ELISA. Intracellular IL-1ra in keratinocytes may influence IL-1 receptor expression (or function) and thus IL-1 responsiveness.

We conclude that UVR-induced ICAM-1 expression in human keratinocytes can be mediated by both TNF-a and IL-1, but that the IL-1 effect is inhibited by endogenous icIL-1ra. TNF-a response is a resproducible individual characteristic of cells from different donors and correlates with the level of UVR-induced ICAM-1.

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PENTOXIFYLLINE (PTX) SUPPRESSES UVB-INDUCED CYTOKINE RELEASE BY KERATINOCYTES
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Currently we could show that the phosphodiesterase inhibitor PTX is able to suppress the effector phase of contact hypersensitivity and irritant reactions. This activity may be explained by the recently described ability of PTX to downregulate the release of tumor necrosis factor α (TNFα) by leukocytes because TNFα is a critical mediator in these events. As keratinocytes are known to be a source of a variety of cytokines like interleukin (IL) 1, IL-6 and TNFα the effect of PTX on the release of these mediators by keratinocytes was studied. Thus several keratinocyte cell lines (A431, KB, HaCaT) and normal human keratinocytes were exposed to UVB-light (FS40) and supernatants tested for IL-1, IL-6 and TNFα levels. UVB-irradiation caused a significant upregulation, while addition of PTX immediately after UVB-exposure resulted in a reduction of IL-1, IL-6 and TNFα release. In contrast, addition of PTX to unirradiated cells slightly increased constitutive cytokine secretion. The inhibitory effect of UVB-mediated cytokine production by PTX was maximally pronounced between 50 and 100 ng/ml, suppression was even observed when PTX was added 1 hr after irradiation. However, treatment of cells with PTX at later time points had no effect. To study whether downregulation by PTX is a general phenomenon keratinocytes were treated with PMA, another well known cytokine inducer. Addition of PTX to PMA treated keratinocytes, however, showed no suppression or an even slightly increased release of IL-1, IL-6 and TNFα. In summary, these data demonstrate PTX as a substance which is able to downregulate UVB-induced cytokine production, a phenomenon which has been so far only described for corticosteroids. The fact that PTX blocks UVB-induced but stimulates PMA-mediated cytokine release supports the concept that PMA and UVB-light work via different pathways.

3

REGULATION OF CYTOKINE GENES INVOLVED IN CYTOKINE NETWORK IN THE ECCRINE SWEAT GLAND.
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Concentrations of interleukin (IL)-1α in human sweat are much higher than that in plasma and are secreted into sweat on a persistent basis, suggesting that sweat IL-1 has biological functions in health and disease. We therefore addressed the questions: is IL-1α synthesized de novo?; which cytokines are present in sweat?; is IL-1α part of a cytokine network?; and what is the pharmacological and molecular basis for the regulation of such a cytokine network? Using Western blot analysis, we demonstrated the presence of IL-6, IL-8, and IL-1α and β in freshly secreted human sweat and in the tissue homogenates as well as in the sweat gland culture medium (both the duct and the coil). Furthermore, mRNAs for IL-1, -6 and -8 were also found in cultured sweat gland cells using Northern blot analysis. This indicates that the sweat cytokines are produced de novo. IL-1α (0.5-1 ng/ml,recombinant or of sweat origin) stimulated mRNA levels for IL-1β, IL-6, and IL-8, peaking at 2-6 h after stimulation, suggesting that these cytokines are part of a cytokine network where IL-1α plays a pivotal role as a master trigger. IL-6, which is mainly regulated by sweat prolactin, was a strong inhibitor of IL-8 mRNA expression while IL-8 had no effect on IL-6 production, indicating that IL-6 is a regulator of IL-8. cAMP strongly inhibited mRNA levels for IL-1α and IL-8, but slightly stimulated IL-6 mRNA. Phorbol ester (TPA) and a Ca-ionophore (A23187) (both of which are mimicked by high [ACh]) weakly elevated IL-1α mRNA at 6 h. The role of IL-1α as a master regulator of the cytokine network in the sweat gland is further supported because sweat IL-1α is the strongest inducer of DNA binding proteins such as CRE (cAMP-binding element), CTF/NF-1 (CCAAT sequence binding element), and NF-κB (κ-light chain enhancer) in the gel-retardation assay of nuclear extracts. We conclude that cytokines in sweat are produced de nova and form a network, where IL-1α regulates the gene expression of other cytokines at the transcription level.

4

CHOLERA TOXIN (CT) AND cAMP UPREGULATE IL-4 GENE EXPRESSION IN TH0 CELLS.
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T cell-derived IL-4 plays a critical role in immunologic abnormalities associated with atopic allergy such as excessive IgE production. Because Th1 and Th2 cells may represent the extreme ends of a developmental spectrum of T helper cells with a fixed lymphokine pattern and because human lymphokine producing T cell clones rarely show a pure Th1 or Th2 profile, we focused on T cells with an unrestricted and nonfixed lymphokine pattern (Th0). In these cells, we investigated the effect of CT and cAMP on IL-4 expression. Since these reagents have been reported to exert dramatic effects on IL-2 production, the effect on the IL-4 response was compared to IL-2. Th0 cells (IL-2+IFN-γ+IL-4+IL-5+) were generated from freshly isolated small resting CD4+ T cells by incubation with irradiated spleen cells, ConA and IL-2 for 7 days. Cells were then washed and restimulated with ionomycine, PMA and either CT or dibutyryl-cAMP. Accumulation of IL-4 and IL-5 in the supernatant was increased in a dose-dependent fashion by both CT and cAMP. IL-2 was inhibited while the effect on IFN-γ was inconsistent. IL-4 mRNA accumulation as determined by PCR was increased by CT and cAMP whereas IL-2 mRNA was diminished. Electrophoretic mobility shift assays showed a decreased IL-2 NF-AT complex formed with extracts from cells treated with CT when compared to cells stimulated with ionomycine and PMA alone. This is the first demonstration of the CT sensitivity of the IL-2 NF-AT complex. The NF-AT site has previously been shown to be important for the upregulation IL-2 expression. Complexes obtained on a canonical AP1 site, on the NF-IL-2A site and on a canonical CREB site were not affected by CT treatment. However, we identified on the P element of the IL-4 promotor an NF-AT-like complex that is also CT sensitive. In summary, our experiments demonstrate that CT and cAMP upregulate IL-4 production by increasing the steady state levels of IL-4 mRNA. The control of IL-4 expression by regulatory nuclear factors remains to be elucidated. The decrease of IL-2 production by CT was correlated with decreased mRNA levels and decreased protein complexes binding to the NF-AT site that profoundly influences IL-2 expression.

5

Parasite Infections Break T Cell Tolerance - a Model for Autoimmunity Induced by Infectious Diseases?
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The origine of autoimmune diseases remains an enigma since non deleted, potentially autoreactive T cells are normally tolerized. Potentially autoreactive, but tolerized T cells could be considered as a source of autoimmunity only if tolerance can be reversed under physiologic conditions. T cell tolerance has previously been reported to affect exclusively the interleukin-2 (IL-2) pathway and it has been suggested that tolerant T cells could be interleukin-4 (IL-4) producing or Th2 cells. To characterize more precisely the mechanisms that induce and reverse T cell tolerance we investigated the state of tolerance established in CD4+ T cells that follows after priming of mice with staphylococcus enterotoxin A or B (SEA or SEB). CD4+ T cells from animals primed with repetitive injections of SEA or SEB (100 μg) were incapable of producing IL-2, IL-3, IL-4 or intrerferon-γ. Thus the state of tolerance induced by the injection of superantigens closely reflects the state of selftolerant CD4+ T cells and does not represent a differentiation of IL-2 producing CD4+ T cells toward a Th2 phenotype. Infection of SEB/SEA-tolerant animals with parasites could break T cell tolerance, by induction of CD4+ T cells that produced IL-4 in response to the tolerizing antigen. However, the induction of the Th2 phenotype in tolerant CD4+ T cells required polyclonal activation and expansion of the tolerant T cell population. Thus the infection did not circumvent T cell tolerance but reversed tolerance by activation of a silenced lymphokine pathway. These findings should further our understanding of autoimmune phenomena that frequently complicate parasitic diseases such as malaria, leishmania or trypanosomiasis, after a strong polyclonal T cell activation.

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KERATINOCYTE-DERIVED TGFβ SERVES AS A GROWTH SUPPRESSIVE FACTOR FOR MOUSE DENDRITIC EPIDERMAL T CELLS.
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Dendritic epidermal T cells (DETC) are γδ T cells that reside normally in mouse epidermis. We have observed that DETC growth is supported by IL-2 produced by DETC and by IL-7 produced by keratinocytes (KC). In this study, we sought to identify an epidermal cytokine that might down-regulate their growth. Transforming growth factor β (TGFβ) is a family of structurally homologous dimeric proteins (TGFβ1 through TGFβ5), which display potent immunosuppressive activities. We observed TGFβ1 mRNA expression in Pam 212 KC and in freshly isolated mouse epidermal cells (Northern blotting). TGFβ1 bioactivity (0.1 ng/ml range) and immunoreactivity were detected in Pam 212 supernatants (CCL64 bioassay and antibody blocking). We then tested whether rTGFβ1 affects DETC growth. Proliferative responses of the 7-17 DETC line and freshly isolated DETC to mitogens (Con A, immobilized anti-CD3, or PMA plus ionophore) were blocked (80%) by 0.1-1 ng/ml rTGFβ1 (^{3}H-thymidine incorporation). TGFβ1 also blocked Con A-driven entry of DETC into S phase (cell cycle analysis). With respect to its mechanism of action, TGFβ1 blocked almost completely the production of IL-2 (ELISA) and the expression of high affinity IL-2 receptors (FACS and binding assay), both of which are early events ordinarily induced by Con A. Furthermore, proliferative responses of preactivated DETC to rIL-2 or to rIL-7 were inhibited (30-50%) by TGFβ1 as well as by rTGFβ2, β3 or β5. These results indicate that TGFβ suppress mitogen-induced DETC growth by down-regulating IL-2 production and IL-2 receptor expression. TGFβ also inhibits DETC responsiveness to IL-7, suggesting that KC-derived IL-7 and KC-derived TGFβ regulate DETC growth in vivo by reciprocal mechanisms.

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KERATINOCYTE-DERIVED IL-7 SERVES AS A GROWTH FACTOR FOR DENDRITIC EPIDERMAL T CELLS. H Matsue, PR Bergstresser, A Takashima. UT Southwestern, Dallas, TX, USA, and Hokkaido Univ., Sapporo, Japan

Dendritic epidermal T cells (DETC) are $CD3^+/CD4^-/CD8^-/\gamma\delta$-$TCR^+$ T cells that reside normally in mouse epidermis. To address mechanisms by which DETC survive in situ, we examined 12 different cytokines that are expressed at mRNA levels in mouse epidermis for their capacities to support the growth of the 7-17 DETC line. In addition to IL-2, which is secreted by DETC, IL-7 also produced vigorous proliferation. IL-7 and IL-2 were effective at comparable concentrations (0.5-2.5 ng/ml); IL-7 promoted continuous DETC growth, whereas IL-2 responses were more rapid and transient. Moreover, IL-7 augmented Con A-induced proliferation of FACS-purified $Thy\text{-}1^+$ epidermal cells (i.e., freshly-isolated DETC). IL-7-driven proliferation was blocked by anti-IL-7, but not by anti-IL-2, while IL-2-driven proliferation was blocked only by anti-IL-2, indicating that DETC respond to IL-7 and to IL-2 by independent mechanisms. "Activated" DETC (cells stimulated with Con A 2-5 days before), but not "resting" DETC (stimulated 10-20 days before) proliferated to IL-7, indicating a dependence upon the state of cell activation. Resting DETC, however, did respond to IL-7 by showing prolonged survival; cells maintained for 13 days with IL-7 alone remained functionally active, as assessed by their proliferative response to mitogens. Mouse epidermal cells and Pam 212 keratinocytes (KC) both expressed IL-7 mRNA constitutively, as demonstrated by RT-PCR analyses. Furthermore, IL-7 production by KC was demonstrated using a bioassay; Pam 212 culture supernatants promoted the growth of 7-17 DETC, and this activity was diminished with anti-IL-7. These results indicate that IL-7 serves as a growth factor for DETC and that KC produce IL-7 in biologically relevant amounts. We propose that IL-7-mediated interaction between KC and DETC represents one mechanism by which the indefinite residence of DETC in mouse epidermis is sustained.

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PROOPIOMELANOCORTIN PEPTIDES ARE POTENT MODULATORS OF HUMAN IgE SYNTHESIS. *I.Aebischer, +E.Schauer, #A.Schwarz, #T.Schwarz, *B.M.Stadler, #T.A.Luger, *Inst. of Clin.Immunology, Inselspital Bern, Switzerland; +Dept. of Dermatology, Univ. of Vienna, Austria; #Ludwig Boltzmann Inst. of Cellbiol. and Immunobiol., Dept. of Dermatology, Univ. of Münster, Germany.

In accordance with the postulated relationship between the neuroendocrine and the immune system it turned out that pituitary glands derived hormones such as proopiomelanocortin (POMC) yielding αmelanocyte stimulating hormone (αMSH), adrenocorticotropin (ACTH) and others are produced by many different cells including lymphocytes and keratinocytes. In addition, it became apparent that among the immunomodulating capacities of POMC-peptides they are involved in the upregulation of IgE production by human PBMC. Therefore, the present study was performed to further investigate the underlying mechanisms of IgE regulation by POMC-peptides. ACTH or αMSH (10^{-7} to 10^{-12} M) were added to POMC in combination with IL-4 or αCD40mAb and IgE-synthesis was measured after two weeks. In addition, IFNγ or TGFß known to antagonize IL-4 effects were used. ACTH and αMSH at physiological concentrations (10^{-10} M) significantly increased IgE-production induced by IL-4 and anti-CD40mAb. Upon addition of IFNγ or TGFß the stimulatory effect of ACTH was abolished. Moreover, ACTH was unable to influence IgE-synthesis by the myeloma cell line U266 indicating that ACTH probably modulates IgE-synthesis by influencing the cytokine production. To further address this question the effect of POMC-peptides on IFNγ production was investigated. αMSH at physiological concentrations (10^{-11} M) significantly downregulated IFNγ release as well as IFNγmRNA expression by ConA stimulated human PBMC. These findings indicate that POMC-peptides are involved in IgE regulation possibly by modulating the cytokine microenvironment and thereby may play an important role in the pathogenesis of allergic diseases.

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INTERLEUKIN 5 SYNTHESIS OF ACTIVATED EOSINOPHILS IN THE LESIONS OF PATIENTS WITH ATOPIC DERMATITIS. Yoichi Tanaka*, Emmanuel Delaporte**, Monique Capron***, *Department of Dermatology, Nagasaki University School of Medicine, Nagasaki, Japan, **Service de Dermatologie A, Hôpital Cl.Huriez, Lille, France, ***the Centre d'Immunologie et Biologie Parasitaire, INSERM U167 Institut Pasteur, Lille, France

In atopic dermatitis(AD), infiltrating eosinophils are in an activated state in view of EG2 positive staining. Bearing in mind that interleukin 5(IL-5) has a wide range of effects on eosinophils, IL-5 may account for eosinophil infiltration and activation of eosinophils in AD. However, the cellular source of IL-5, specially in the lesions of atopic diseases, remained to be elucidated. In an attempt to elucidate the IL-5 mRNA expression, the translation to IL-5 protein and a nature of the IL-5 synthesizing cells, combination of in situ hybridization with ^{35}S-labelled IL-5 RNA probe and EG2 staining, and double-immunostaining with anti-IL-5 monoclonal antibodies(mAb) and polyclonal anti-Eosinophil Cationic Protein(ECP) were performed. Part of EG2 positive cells expressed IL-5 mRNA, and double stained cells with anti-ECP and anti-IL-5 mAb were observed. Besides, mononuclear cells, presumably lymphocytes, expressed IL-5 mRNA and accumulated IL-5 protein. Taken together, our results indicated that activated eosinophils, in addition to lymphocytes, can elaborate IL-5, and suggested that eosinophils expand and become activated in autocrine fashion.

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CYTOKINE GENE ACTIVATION ACROSS THE ADVANCING EDGE OF PSORIATIC PLAQUES. John B Mee, Michael J Cork*, Francesco S di Giovine, Gordon W Duff and Stanley S Bleehen*, Sections of Molecular Medicine and *Dermatology, Department of Medicine, University of Sheffield, Sheffield, UK

Numerous abnormalities of primary and secondary cytokine production have been demonstrated in psoriasis but the identity of the initiating event(s) is not clear. This study provides a new approach to investigate the initiating and subsequent events that produce psoriatic lesions. Across the active edge/non-lesional boundary of psoriatic plaques, six 3mm punch biopsies were taken. Semi-quantitative profiles for cytokines and related immunopeptides have been established using reverse transcription polymerase chain reaction (RT-PCR) to assay, initially, IL-1α, IL-1β, IL-1 receptor type I, IL-1 receptor antagonist (IL-1ra) and IL-8 mRNA levels, relative to a novel cell-cycle independent housekeeping gene transcript, L41. Immunohistochemistry was also performed on these samples.

A gradient of IL-1α mRNA and protein expression has been established from high levels in normal skin to lower levels in psoriatic skin. In contrast, the levels of IL-1β mRNA were lower in non-lesional skin and highest on the edge of the psoriatic plaque, verified by immunohistochemistry. IL-1 receptor type I mRNA and protein levels were found to be substantially increased, both in lesional and non-lesional skin compared with normal individuals. Of interest, raised IL-1 receptors occurred in non-lesional skin up to 6cm from the edge of the lesion. IL-1ra mRNA appeared to be unchanged across the lesional/non-lesional boundary, contrasting sharply with the decreased levels of IL-1ra protein in lesional skin. IL-8 mRNA was increased in the psoriatic lesion.

Our results are consistent with increased IL-1 biological activity in the psoriatic lesion. This technique identifies candidate cytokines for genetic investigations in psoriasis and suggests that IL-1 receptor type I may be such a gene.

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NEUTROPHIL-ACTIVATING CHEMOKINES IN PSORIASIS. Jens-Michael Schröder, Michael Sticherling, Joachim Bartels and Enno Christophers, Department of Dermatology, University of Kiel, Kiel, Germany

In order to identify stimuli possibly responsible for neutrophil immigration in psoriatic lesions we recently purified ten biochemically different neutrophil chemotactic proteins from psoriatic scales. By amino acid sequence analysis apart from four IL-8-related cytokines another structurally related neutrophil attracting chemokine could be identified, which is identical with the so-called "16 kD form of melanoma growth stimulatory activity, MGSA"/gro-α. The aim of this study was the structural characterization of other neutrophil chemotactic cytokines present in psoriatic scales. Two yet unidentified chemokines could be separated from IL-8 depleted psoriatic scale extracts by the use of different HPLC-techniques. Aminoterminal amino acid sequence analyses revealed the same sequence Ala-Ser-Val-Ala-Thr-Glu-Leu-Arg-X-Gln-X... for both neutrophil chemotactic chemokines, which both showed single bands upon SDS-PAGE analyses near 8 kD, however could be separated by reversed phase HPLC. This sequence is identical with that published for the chemokine gro-α. These findings indicate that psoriatic scales contain at least three different forms of gro-α. Another neutrophil chemotactic cytokine present in psoriatic scales shows a unique chromatographic behavior, which is identical with that published for the epithelial cell-derived neutrophil chemotactic chemokine ENA-78. We conclude from these findings that apart from IL-8 three different forms of gro-α represent the major neutrophil chemotactic cytokines present in psoriatic scales, whereas the chemokine ENA-78 could be present also. Other neutrophil chemotactic chemokines such as NAP-2 or gro-ß or gro-γ appear to be less important as neutrophil attractants in psoriatic lesions.

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ASSOCIATION OF CUTANEOUS AND SYSTEMIC FORMS OF LUPUS ERYTHEMATOSUS TO GENETIC VARIATIONS OF THE GENES OF TUMOR NECROSIS FACTOR-α AND LYMPHOTOXIN. Dorothe Schmidt, Gerald Messer, Sabine Franz, Monika Walchner, Hans C. Schuppe[1], Elisabeth H. Weiss[2], Gerd Plewig and Peter Kind, Department of Dermatology, L-M-University of Munich; [1]Department of Dermatology, University of Düsseldorf; [2]Institute for Anthropology and Human Genetics, L-M-University of Munich; W-8000 München 2, F.R.G.

Tumor Necrosis Factor-α (cachectin, TNF-α) and -ß (lymphotoxin-α, TNF-ß) have been characterized and mapped in 1.2 kb distance *in tandem* within the human major histocompatibility complex (MHC). A third functional TNF gene called lymphotoxin-ß (LT-ß) was identified and located 2 kb centromeric of TNF-α. The LT-ß gene encodes the transmembrane chain of lymphotoxin and acts as a heterotrimer with TNF-ß (LT-α). Systemic lupus erythematosus (SLE) is associated with the HLA-haplotype A1, -B8, -DR3. Stimulated PBMC of healthy individuals and SLE patients with HLA-DR3 have been shown to produce higher levels of the cytokines TNF-α and TNF-ß *in vitro*. Restriction fragment length polymorphisms (RFLP's) of the genes for TNF-α (TNF1, TNF2) and TNF-ß (TNFB*1, TNFB*2) are found in linkage disequilibrium with HLA-B8, -DR3. The allele TNFB*1 is significantly increased in SLE. In order to investigate whether genetic differences in the TNF genes could play a pathophysiological role, we analyzed different TNF alleles of several subforms of cutaneous LE and SLE.

Genomic DNA of 80 LE patients which are clinically and histologically characterized as either systemic LE (12) or cutaneous subforms as subacute cutaneous (SCLE 19), discoid (DLE 37), LE tumidus (LET 11), LE profundus (LEP 1) was isolated. The TNF-α promoter and the first intron of LT-α were amplified by the polymerase chain reaction (PCR) and analyzed with the respective restriction endonuclease or typed by single strand conformation polymorphism (SSCP). No strong deviations of the allele frequencies of the TNF-α promoter variation TNF2 was found among the cutaneous subforms (N=67, 0.25), DLE (0.26), SCLE (0.26), LET (0.23). The random controls (0.19) presented a lower allele frequency, only the group of SLE patients had a reduced frequency of TNF2 (0.16). Typing of the rare allele TNFB*1 of the LT-α/TNF-ß Nco I RFLP revealed an elevated frequency of TNFB*1 in SLE (N=12, 0.46; Bettinotti et al. 1993, Immunogenetics 1993, N=173, 0.39), DLE (0.38), SCLE (0.37) compared to the total group of cutaneous LE patients (0.34) and controls (N=179, 0.33). In contrast, LET (0.17) had a reduced frequency of TNFB*1. Phenotype and allele frequencies within cutaneous LE do not differ from the controls. In SLE patients an association is found with a variant TNF-ß gene (TNFB*1), possibly secreting higher amounts of TNF-ß. Thus this T cellular cytokine could play a role in the so far unknown pathogenesis of SLE.

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IL-10 IS A POTENT REGULATOR OF IL-8-STIMULATED IL-8-PRODUCTION BY HUMAN T LYMPHOCYTES. C. G. Larsen, B. Deleuran, M. Lund, B. Gesser, Department of Dermatology, Marselisborg Hospital, University of Aarhus, Aarhus, Denmark.

The neutrophil and T lymphocyte chemotactic cytokine, IL-8, is believed to play a pathophysiological role in different inflammatory diseases of the skin, including psoriasis and contact dermatitis. IL-10 is a recently described cytokine, which suppress several functions of the immune system, including cytokine production, class II antigen expression, T cell chemotaxis, etc. We observed that recombinant IL-8 (rIL-8) potently induce its own production by positively selected human $CD4^+$ as well as $CD8^+$ T cells, as judged by 2-dimensional gel analysis of cytosolic proteins, revealing *de novo* production of IL-8 and the IL-8-precursor. Also, conditioned media from overnight cultures of IL-8-stimulated T cell subsets contained increased levels of IL-8 as judged by ELISA, 2 D-gel analysis, including fluorograms (S^{35}-methionin incorporation), showing that rIL-8 stimulate the production, as well as the release of IL-8. The Western blotting analysis showed specific staining using a monoclonal anti-IL-8 antibody. Further, the PCR-analysis demonstrated that rIL-8 significantly and strongly stimulated the production of IL-8-mRNA by T lymphocytes after 12 hours, and that a neutralising IL-8-antibody could completely block the observed IL-8-stimulated IL-8-mRNA production, indicating the specific action of IL-8. Double-staining immunofluorescence showed that the IL-8 producing cells also expressed CD3, thereby confirming that the IL-8 producing cells were T cells. Finally, we observed that rIL-10 (10 to 100 ng/ml), when added to T cells 30 min. before stimulation with IL-8, were able to almost completely block the IL-8 induced IL-8 production, as judged by 2-dimensional gel analysis and by PCR analysis. These results reveal a novel auto-stimulatory and thereby self-enhancing effect of the T cell chemotactic cytokine, IL-8, and suggest that this potentially inflammation-sustaining cytokine-circuit is strongly regulated by IL-10.

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TRANSIENT EXPRESSION OF TYPE II COLLAGEN AT THE MIDDLE STAGES OF CHICK EMBRYONIC AND HUMAN FETAL SKIN DEVELOPMENT. Tatsuya Izumi, Shingo Tajima and Takeji Nishikawa, Department of Dermatology, Keio University School of Medicine, Tokyo, Japan

Type II collagen is the major and unique collagenous component of cartilage and plays a critical role in chondrogenesis during embryonic development. However, it has been demonstrated that type II collagen is much more widely distributed during early development than previously thought. Small amount of type II collagen has been detected in nonchondrogenic tissues. In this study we found type II collagen genes and gene product in the chick embryonic and human fetal skins. Type II collagen mRNA was found to be detected in the epidermis at the stages from day 9 to 15 with a maximum expression at day 11 in the chick embryonic skins using in situ hybridization and RNase protection assay. Immunohistochemical studies using monoclonal type II collagen antibody has demonstrated that type II collagen was present in the subepidermal region at the stages from day 10 to 15 in the chick embryonic skins. Type II collagen gene and gene product in the regions where the feather buds developed started to diminish at the stage of day 12 and those in the regions between feather buds thereafter gradually disappeared. In human fetal scalp, type II collagen was also detected in the subepidermal region at the stages of 17-23 fetal week avoiding the subepidermal region beneath the hair germs, but was not detected in the back skin. These results indicate that type II collagen expression strictly controls the development of feather or hair at a certain stage of both chick embryonic and human fetal skin development.

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REGULATION OF α1 (I) COLLAGEN GENE PROMOTER BY TUMOR NECROSIS FACTOR-α IN HUMAN DERMAL FIBROBLASTS

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The role of cytokines in the regulation of connective tissue metabolism has been increasingly emphasized by elucidation of their specific effects using recombinant gene products. Recent studies have demonstrated that tumor necrosis factor-α (TNF-α) decreases α1 (I) collagen gene expression in cultured human dermal fibroblasts. The purpose of this study was to analyze transcriptional control of the α1 (I) collagen gene by TNF-α by means of DNA mediated transfection experiments using recombinant plasmids in which the promoter region of the human α1 (I) collagen had been fused to the chloramphenicol acetyltransferase (CAT) gene, in human dermal fibroblasts. Approximately 5-fold decreased levels of CAT activity were observed in fibroblasts treated with 10 and 100 ng/ml of TNF-α, when 2300 bp of the α1 (I) collagen promoter gene fused to the CAT gene was transfected. Similar results were also obtained in the fibroblasts, when 800 bp of the α1 (I) collagen promoter fused to the CAT gene was transfected. On the other hand, the levels of CAT activity were unaltered in fibroblasts transfected with the control gene. Our data show that the expression of chimeric collagen CAT gene is strongly inhibited by TNF-α in human dermal fibroblasts, suggesting that a common mechanism inhibits both transfected and endogenous α1 (I) collagen promoters. It is also suggested that TNF-α reduces α1 (I) collagen transcription through at least up to 800 bp upstream of the α1(I) collagen promoter. Work is in progress to analyze regions further downstream to determine which elements are responsible for decreased transcription with TNF-α.

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CHARACTERIZATION OF SPATIAL DISTRIBUTION AND ASPECTS OF REGULATION OF COLLAGEN GENE EXPRESSION IN KELOIDS. Stephan Sollberg (1,2), Elaine M.L. Tan (2) and Jouni Uitto (2), Departments of Dermatology, (1)University of Cologne, Germany, and (2)Thomas Jefferson University, Philadelphia, PA, USA

In keloid tissues active collagen gene expression is localized to the areas containing an abundance of fibroblasts and apparently representing the expanding border of the lesions. Within this zone, microvascular endothelial cells also express type I collagen genes. Transforming growth factor-ß (TGF-ß) protein and mRNA are also detected in areas active in collagen gene expression, including microvascular endothelial cells. In addition, the effects of fibroblast growth factors (FGFs) on collagen expression by cultured keloid fibroblasts are studied. Acidic FGF (aFGF) has little or no effect on collagen synthesis. However, in the presence of heparin, aFGF decreases [3H]hydroxyproline synthesis by 44% to 68%. Basic FGF (bFGF) is effective in suppressing [3H]hydroxyproline synthesis by 50% to 90% without heparin in keloid and normal fibroblast cultures. The mRNA steady-state levels of type I collagen are significantly decreased by aFGF in the presence of heparin as well as by bFGF without heparin. The data suggest that the FGFs are effective in downregulating excess collagen production by keloid fibroblasts.

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REGULATION OF TYPE XV COLLAGEN GENE EXPRESSION BY CYTOKINES. Sirpa Kivirikko, Alain Mauviel, Taina Pihlajaniemi, and Jouni Uitto, Department of Dermatology, Jefferson Medical College, Philadelphia, PA, USA, and Department of Medical Biochemistry, University of Oulu, Oulu, Finland.

Type XV collagen is a ubiquitously expressed nonfibrillar collagen. The gene encoding the α1 chain of type XV collagen was recently cloned from a human placenta cDNA library and encodes a 125 kD polypeptide. Using Northern blot hybridizations, we have examined the regulation of type XV collagen gene expression by various cytokines in dermal fibroblasts in culture. Transforming growth factor-β (TGF-β), a well known activator of extracellular matrix gene expression, strongly elevated type XV collagen mRNA levels in a dose-dependent manner. By contrast, the pro-inflammatory cytokines interleukin-1, (IL-1), and tumor necrosis factor-α (TNF-α), reduced the expression of type XV collagen also in a dose-dependent manner. Furthermore, when fibroblasts were incubated with IL-1 or TNF-α together with TGF-β, the up-regulation of type XV collagen gene by TGF-β was abolished by these two cytokines. It appears, therefore, that the expression of type XV collagen gene can be regulated by various cytokines and this regulation strongly correlates with that of type I collagen gene.

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$CD34^+$ FIBROBLASTS SELECTIVELY DISAPPEAR FROM THE SKIN LESION OF SCLERODERMA. Setsuya Aiba, Hachiro Tagami, Department of Dermatology, Tohoku University School of Medicine, Sendai, 980, Japan.

The etiology of scleroderma is still unknown, although fibrosis is a critical damaging factor. Human fibroblasts have been found to be remarkably heterogeneous functionally and phenotypically. Recently it has become possible to identify different subpopulations of dermal fibroblasts or spindle cells using anti-CD34 and anti-factor XIIIa antibodies. The purpose of this study was to elucidate whether the entire populations of dermal fibroblasts or only a subpopulation of cells are involved in development of the fibrosis in scleroderma. We compared the staining pattern of several antibodies to human cutaneous fibroblasts or spindle cells in paraffin-embedded tissue sections and cryostat sections prepared from the skin lesions of 30 patients with scleroderma, including PSS and morphea, and 15 with other collagen diseases, and normal skin of 17 subjects. We used the antibodies to CD34, factor XIIIa, human type I procollagen aminopeptide, and proline-4-hydroxylase. The number of positive cells were counted in three randomly chosen square fields (1,000 μm x 700 μm) in the reticular dermis.

$CD34^+$ cells were few or absent in the lesions of scleroderma (0.2 ± 0.8), while a number of $CD34^+$ cells were found in the lesions of other collagen diseases (53 ± 29) as well as in normal skin (34 ± 12). In contrast, a large number of factor $XIIIa^+$ (27 ± 16), type I procollagen aminopeptide$^+$ (11 ± 18) or proline-4-hydroxylase$^+$ cells were noted in the lesions of scleroderma. Even in the clinically uninvolved skin of patients with scleroderma, $CD34^+$ cells were less frequent and more slender than those in normal skin.

Immunohistological staining with anti-CD34 and other antibodies to dermal spindle cells or fibroblasts demonstrated a selective disappearance of $CD34^+$ fibroblasts from the lesions of scleroderma. It suggests that $CD34^+$ cells might be important target cells in the autoreactive phenomenon in scleroderma.

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A PATIENT WITH EHLERS DANLOS SYNDROME TYPE VI IS A COMPOUND HETEROZYGOTE FOR THE LYSYL HYDROXYLASE GENE. Heather N. Yeowell, Van Ha, and Sheldon R. Pinnell, Duke University Medical Center, Durham, NC.

We have recently cloned and sequenced a full length cDNA for lysyl hydroxylase (LH) from cultured normal human dermal fibroblasts. This has enabled us to characterize the mutations in the cDNAs for LH in fibroblasts from one patient (AT750) with Ehlers Danlos Syndrome Type VI (EDS VI) which may be responsible for the patient's decreased LH activity (25% of normal). Intermediate levels of LH activity were measured in fibroblasts from the patient's father (52%) and mother (86%). Northern blot analysis of total RNA from fibroblast strain AT750 showed an abnormally migrating 3.4 Kb LH mRNA. Using RT-PCR techniques, we amplified partial and full length LH cDNAs from these dermal fibroblasts using PCR primers with linked Eco RI recognition sequences. Following ligation and cloning, sequence analysis of single stranded DNA prepared from 3 full length clones and 10 partial clones revealed the existence of 2 equally distributed populations of cDNAs for LH in the AT750 cell line. Both alleles revealed some significant sequence changes which were different in each allele. One allele, (allele 1) differed from the normal sequence by a triple base deletion between nts 1618-1620 (GAG) which would result in the loss of residue Glu_{532}. In addition, allele 1 contained 2 single base changes giving rise to Ala_{90}→Thr and Ala_{120}→Ser, as well as 1 conservative base change at nt 1656. The other allele (allele 2) differed from normal human LH cDNA by 3 base changes, two of which are conservative and in a variable region. The third change is a G→A substitution at nt 2056 that would give a significant Gly_{678}→Arg change in a highly conserved region which may function as the catalytic domain of LH. This study represents the first example of compound heterozygosity for the LH gene in an EDS VI patient and the mutations identified in both alleles may each contribute to the disease phenotype of this patient.

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CYTOKINES ACTIVATE TYPE-I COLLAGEN GEL CONTRACTION REORGANIZED BY DERMAL FIBROBLASTS CO-CULTURED WITH VASCULAR ENDOTHELIAL CELLS. Ken-Ichi Toda,Chiung-Shan Chen,and Sadao Imamura, Dept. of Dermatol., Kyoto Univ., Kyoto Japan

In cutaneous wound repair, wound contraction is of major clinical importance in reducing the size of the wound. Although it is well recognized that dermal fibroblasts play major roles on the wound contraction, it is still unclear how these fibroblasts-induced wound contraction is related to the other dermal resident nonmuscle cells. In the present study, by using the in-vitro 3 dimensional hydrogen type I collagen gel system in which both human dermal fibroblasts(F) and/or human umbilical vein derived endothelial cells(E) were co-cultured, we studied the gel contraction activities. In serum free DMEM media, either F-or E-reorganized collagen gel did not significantly contract in 48 hrs. The contraction of both F and E mixed cultured collagen gel was not notable. However, the supplementation of such cytokines as TNF-α or IL-1 in the media promoted the mixed-gel contraction and the gel thickness was decreased to 60 % of the original gel thickness in 48 hrs. TNF-α or IL-1 did not promote the contraction of the either F- or E- collagen gel. The conditioned media of the E-collagen gel stimulated by the cytokines also promoted the F-collagen gel contraction. Anti-integrin β1 antibodies remarkably suppressed the gel contraction. Anti-endothelin 1(ET-1) antibodies also partially suppressed the contraction. These results suggest that (1)in this in vitro wound contraction model, the cytokines such as TNF-α or IL-1, stimulate vascular endothelial cells to secrete the factor(s) which promote the wound contraction, and(2)the contraction is mediuated by β1 integrins expression on fibroblasts, and(3) ET-1 is a effective wound contraction factor.

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HEPARIN AND HEPARANSULFATE MODULATE CELL-MATRIX INTERACTIONS OF FIBROBLASTS AND ENDOTHELIAL CELLS IN VITRO AND INTERACT WITH SPECIFIC BINDING SITES. Th. Schaefer[1], M. Roux[1], H.W. Stuhlsatz[2], Th. Krieg[1], H. Smola[1], [1]Dept. of Dermatology, University of Cologne, [2]Dept. of Clinical Chemistry, RWTH Aachen, Germany.

During embryonic developement, tissue remodeling and wound healing, several cellular functions have been shown to be modulated by components of the extracellular matrix. We are especially interested in investigating the influence of different GAGs on the interaction of mesenchymal cells with a collagenous matrix. Human dermal fibroblasts (HDF) and human umbilical vein endothelial cells (HUVEC) were embedded into collagen type I/III gels to which different purified GAGs were added. Contraction of the collagen gel was measured after 24 hours and mRNA levels for collagen, collagenase and IL 6 were determined. GAGs did not modulate HDF gel contraction. In contrast, heparin and heparansulfate almost completely inhibited gel contraction by HUVEC. In northern blot experiments HDF collagen type I mRNA was not modulated by GAGs. Collagenase and IL 6 mRNA levels were induced in dermal equivalents while heparansulfate and heparin significantly decreased transcripts. HUVECs were negative for IL 6 mRNA and expressed only few transcripts for collagen type I. In monolayer cultures they constitutively expressed high levels of collagenase mRNA. In collagen gels collagenase transcripts were down regulated. To demonstrate specific binding sites for GAGs we performed binding studies with ^{35}S-labelled heparin. On HDF binding sites displayed a K_D of about 8 x 10^{-8} M whereas HUVEC were shown to have high (1 x 10^{-10} M) and low affinity (4 x 10^{-6} M) binding sites. These results indicate that different GAGs specifically modulate gel contraction. These effects were accompanied by regulation of steady state mRNA levels for collagenase and IL 6 by individual GAGs. Additionally, specific binding sites for heparansulfate and heparin were demonstrated on HDF and endothelial cells.

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DEMONSTRATION OF EARLY ANAGEN-SPECIFIC STIMULATORY FACTORS WITHIN SKIN TISSUE FOR IN VITRO MESENCHYMAL-EPITHELIAL INTERACTION IN COLLAGEN EMBEDDED HAIR FOLLICLE MIXED CULTURE. Mitsuyuki Hotta and Genji Imokawa, Kao Biological Science Laboratories, Tochigi, Japan

Interaction between epithelial and interstitial cells within hair follicle is crucial for the maintenance of hair growth. We recently demonstrated using the mixed culture embedded by collagen matrix that the growth of epithelial cells can be significantly stimulated by the presence of mesenchymal cells. In order to clarify mechanisms underlying the cell-cell association with special reference to hair cycle, we determined whether endogenous stimulatory factors involved in the interaction emerge within skin tissue in relation to hair cycle and characterized the biological significance. Crude skin extracts from rat early anagen skin(5-day-old), mid anagen skin(10-day-old) and late anagen skin(15-day-old) were individually fractionated by gel filtration chromatography and tested for the stimulatory effect on DNA synthesis of epithelial cells in the mixed culture system. Assay of the chromatographic fractions revealed that whereas in the both mid and late anagen skin extracts there was no active fractions with the stimulatory activity, the early anagen skin extract contained definite stimulatory factors with molecular weight of approximately 20,000. Study using dorsal skin extracts from the third hair cycle rat also demonstrated that stimulatory activity was mainly associated with the fractions obtained from the early anagen skin(49- to 56-day-old), with the same molecular weight, while the corresponding fractions from the late anagen skin(59- to 70-day-old) exhibited an age-dependent decline in the stimulatory activities, with non-stimulated level attained at 70-day-old. These findings suggest that the hair cycle is primarily associated with the production of early anagen-specific factors which activate mesenchymal-epithelial interaction.

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IMMUNOHISTOCHEMICAL AND MORPHOLOGICAL ALTERATIONS IN EPIDERMAL BASEMENT MEMBRANE IN SKIN CANCERS STUDY USING CRYOFIXED, FREEZE-DRIED AND PARAFFIN-EMBEDDED SKIN SECTIONS. Yuko Onodera[1, 2], Hiroshi Shimizu[1], Irene M. Leigh[3], Makoto Sugawara[2] and Takeji Nishikawa[1], 1: Department of Dermatology, Keio University School of Medicine, Tokyo, Japan. 2: Department of Dermatology, Keiyu Hospital, Kanagawa, Japan. 3: London Hospital, London, U.K.

High-quality immunohistochemistry is essential for the study of epidermal basement membrane (BM) proteins, some of which play important roles in invasion and metastasis of skin cancers. Conventional chemical fixation and paraffin embedding sections (P-sections) preserve morphology well, however, they destroy the antigenicity of many proteins in the tissue sample. On the other hand, fresh frozen sections can preserve the antigenicity, but provide poor morphological preservation. To overcome this dilemma, fresh skin specimens were cryofixed in liquid isopentane (-160 ℃) and freeze-dried at -40 ℃, 10^{-2} atmospheric pressure, then embedded in paraffin. These cryofixed, freeze-dried and paraffin-embedded sections (CF-sections) preserved morphological details as perfectly as P-sections. The antigenicity of various BM antigens, including type VII (LH7.2) and type IV collagen, GB3 antigen, LH39 antigen, laminin and bullous pemphigoid (BP) antigen, was well preserved.

Using CF-sections, we studied BM in patients with Bowen's disease (n=11), Bowen's carcinoma (n=4) and squamous cell carcinoma (n=8). In all three conditions, all antigens were preserved in the portion with few atypical tumor cells and precise arrangement of basal cells. Type IV collagen and laminin showed a loss of reactivity in the area of thickened epidermis with tumor cells cells and disordered arrangement of basal cells. In the area of more distinct thickening and elongation of the epidermis, BP antigen was also negative. LH7.2, GB3 and LH39 antigens were disrupted in the area of tumor cell extension into connective tissue.

CF-sections fulfill the requirement of preservation of both antigenicity and morphology. This method may provide a powerful tool for the precise study of invasion of early skin cancers.

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INITIATION OF THE LAMININ CHAIN ASSEMBLY REQUIRES A SHORT SPECIFIC SEQUENCE LOCATED AT THE END OF THE LONG ARM OF EACH CHAIN. Atsushi Utani*, Motoyoshi Nomizu*, Katsunori Fukuda*, Rupert Timple#, Peter Roller**, Yoshihiko Yamada*, National Institute of Dental Research, National Cancer Institute**, NIH, Bethesda, MD, USA, Max-Planc-Institut#, Munich, Germany

Laminin, a glycoprotein in basement membranes, has diverse biological activity including promotion of cell adhesion, migration, differentiation and growth. Laminin is a heterotrimer in which three chains are assembled into triple stranded coiled-coil alpha-helical structure. There are at least 8 genetically distinct chains which form different isoforms of laminin. The mechanism of the laminin chain assembly was studied by affinity column assay and in vitro reconstitution using various recombinant chains. A 10-amino acid sequence near the C-terminal end of the B2 chain was required to initiate the heterodimer formation with B1 chain and a short stretch of sequence of B2 chain proximal of this sequence was needed to form the heterotrimer with the B1 and merosin chains. Recombinant chains of keratinocyte or epithelial cell specific kalinin B2 chain could not interact with laminin B1 chain or merosin chains, suggesting that ionic interaction played a critical role for the initiation and specificity of laminin chain assembly. Deletion mutation analysis revealed that a 19-amino acid sequence located near the end of the long arm of merosin was crucial for the heterotrimer formation. These results indicated that a short specific sequence of each chain was required to initiate the laminin chain assembly which subsequently proceeded from C- to N-terminus.

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HUMAN KERATINOCYTES SYNTHESIZE LINKIN (MICROTHREAD-LIKE PROTEIN).
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Linkin is a newly described, non-collagenous, connective tissue component within microthread-like fibers localized within the papillary dermis. Ultrastructural studies have demonstrated that microthread-like fibers link three important dermal structures: anchoring fibrils, microfibrils and interstitial collagen bundles.

In this study, we examined whether human keratinocytes (HK) could synthesize linkin. HK cultures were stained with anti-linkin monoclonal antibody, B3a. Linkin was expressed as faint intercellular fluorescence which increased in intensity when TGF-β (10 ng/ml) was added to the cultures. Western blotting of culture medium proteins or cell monolayer proteins with B3a demonstrated that linkin was synthesized within the cells and transported extracellularly. Immunoprecipitation of proteins from ^{35}S-methionine labeled HK cultures with B3a also demonstrated linkin in both the cell layers and secreted medium proteins. Suction blister roofs were raised in five normal adult volunteers and the epidermal proteins extracted in 2 % SDS, 0.125 M Tris-HCl, pH 6.8.The extracted proteins were subjected to Western blot analysis with B3a and control antibody. B3a labeled the 82 kDa linkin band in all five subjects.

We concluded that linkin is synthesized by HK in culture and is also a constitutive protein within human epidermis. Further, the expression of linkin in human keratinocytes is enhanced by TGF-β.

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KERATINOCYTES AND FIBROBLASTS PRODUCE A NOVEL ANCHORING FIBRIL-ASSOCIATING PROTEIN.
Yuji Horiguchi, Masamichi Ueda, Norihisa Matsuyoshi, Kenzo Takahashi, Jo-David Fine and Sadao Imamura, Department of Dermatology, and Institute for Virus Research, Kyoto University Faculty of Medicine, Kyoto, Japan, and Department of Dermatology, University of North Carolina, Chapel Hill, NC, USA

A recently established monoclonal antibody (2.9A, IgM class), produced by immunization of a mouse with human amniotic membrane, reacted to the basement membrane zones (BMZ) of human skin epidermis and vasculatures. Immunoelectron microscopy revealed that a 2.9A antigen along the epidermal BMZ was located at the dermal tips of the anchoring fibril. A preliminary study disclosed that the uninvolved skin of a patient with recessive dystrophic epidermolysis bullosa (DEB) lacked this antigen along the epidermal BMZ with the preservation along the vascular BMZ, but that the dominant DEB skin showed preserved antigen along the epidermal BMZ as well as vascular BMZ. An immunoprecipitation study showed a specific band with a molecular weight of 110kDa from a placental extract under a reduced condition. The transformed human epidermal cell line (HSC-1) was found to produce this protein in the perinuclear cytoplasm showing a granular distribution pattern, distinguishable from that of type VII collagen. The cultured fibroblasts also produced this protein. These findings suggest that the 2.9A monoclonal antibody, recognizing an anchoring fibril-associating protein, is of a diagnostic value in DEB, and is useful for investigating the connection of type VII collagen and other dermal components.

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MOLECULAR EPIDEMIOLOGIC ANALYSIS OF MOLLUSCUM CONTAGIOSUM.
Hiroko Yamashita, Tomoko Uemura and Makoto Kawashima, Department of Dermatology, Tokyo Women's Medical College, Tokyo, Japan.

Molluscum Contagiosum (MC) is a common skin disease among children caused by the MC virus (MCV). MC is also found, though less frequently, in adults with unusual clinical features. Recent studies have revealed that MCV DNAs can be classified into two major groups by their restriction enzyme cleavage patterns. The purpose of this study is to examine MCV types found in Japan and the relationship between MCV types and the clinical features, age, sex, and complications of the patients.

The specimens were collected from 131 cases of Japanese children and 40 adults (male 16, female 24). Total DNAs were extracted and digested with restriction endonucleases (BamHI, HindIII and ClaI, respectively), then electrophoresed in agalose gels. The comparative analysis of cleavage patterns of MCV DNAs revealed six different patterns, which were then classified into two major types (MCV 1, MCV 2) according to the presence of common fragments. MCV 1 was detected in 98% of children, in 92% of females, and less frequently in male patients 56%. MCV 2 was found rarely in children and females adults (2% and 8%, respectively). In contrast, MCV 2 was much more frequently found in male adults 44%, and in 3 of 4 HIV positive patients.

These results may suggest that MC in children and female adults is commonly caused by MCV 1, which is transmitted among them, and that a fairly large number of MC cases in male adults are associated with MCV 2, which is transmitted by a different infection route, probably as an STD.

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ELAM-1 is one of the predominant molecules by which ATL cells adhere to endothelium.
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Adult T cell leukemia(ATL) is characterized by frequent skin infiltration compared with other leukemia/lymphomas. To evaluate the mechanism by which ATL cells infiltrate, we have paid attention to the adhesion of ATL cells to endothelial cells. Using 5 HTLV-I-infected T cell lines, we studied the expression of adhesion molecules by flowcytometric analysis and the properties of cell adhesion to cultured human umbilical vein endothelial cells(HUVEC) in the presence of blocking MoAbs against lymphocyte function-associated antigen-1(LFA-1), very late antigen-4(VLA-4) and endothelial cell leukocyte adhesion molecule-1(ELAM-1). LFA-1 and VLA-4 were expressed on 2 and 2 cell lines, respectively. MoAb against LFA-1 did not diminish the adhesion of LFA-1-positive cell lines to IL-1-activated HUVEC while MoAb against VLA-4 diminished the adhesion of 2 VLA-4-positive cell lines to IL-1-activated HUVEC. The expression of HECA-452Ag(CLA:cutaneous lymphocyte-associated antigen) was detected in 5 cell lines. MoAb against ELAM-1 diminished the adhesion of 4 HECA-452-positive cell lines to IL-1-activated HUVEC. In 4 HTLV-I-infected T cell lines, significant adhesion to HUVEC remained even after LFA-1, VLA-4 and ELAM-1-mediated adhesion was blocked. This adhesion pathway was also detected in 2 cell lines which did not express LECAM-1 and CD44. In contrast, 5 HTLV-I-uninfected T cell lines had no expression of HECA-452Ag and no ELAM-1-mediated pathway of adhesion to HUVEC. Additionally, we also detected ELAM-1 expression on the endothelium at skin lesions by immunohistochemical study in ATL patients and ELAM-1-mediated adhesion pathway between leukemic cells and IL-1-activated HUVEC. These data suggest that ELAM-1-CLA-mediated adhesion pathway plays an important role in the adhesion of HTLV-I-infected T cells to endothelial cells and that undefined molecules may be involved in this adhesion.

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DIRECT DETECTION OF SPLICED HIV-1 mRNA IN EPIDERMAL LANGERHANS CELLS OF HIV-1 SEROPOSITIVE PATIENTS.
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We and others have shown in the past that human epidermal Langerhans cells (LC) are infected by HIV-1 *in vivo* by the demonstration of (1) viral proteins in LC *in situ*, (2) viral DNA in epidermal cells enriched for LC and (3) by budding of HIV from the surface of LC *in situ*. In this study we attempted to detect the expression of viral regulatory (*tat, rev, nef*) and structural genes (*env*) by epidermal cells (EC) highly enriched for LC from HIV-1 seropositive patients. Total cytoplasmic RNA extracted from both LC purified via the CD1a antigen using magnetic beads and from LC depleted EC was converted to cDNA. Primers for detection of HIV-1 specific messages by the polymerase chain reaction (PCR) method were constructed to amplify defined fragments of spliced HIV-1 mRNA. PCR products were analysed on a 2% agarose gel, blotted onto a nylon filter and hybridized with radioactive oligonucleotide probes to ensure specificity. Using this method we detected mRNA for HIV-1 regulatory and structural genes in EC highly enriched for LC but not in LC depleted EC in 2 of 3 patients tested. To test for contaminating T cells in our EC preparation, we performed reverse PCR for a T cell specific mRNA i.e. the CD3 delta chain. Since, in contrast to the expression of HIV-1 mRNA, the amount of T cell specific mRNA (CD3 delta chain) as detected by reverse PCR proved to be significantly greater in EC depleted of LC than in EC enriched for LC, we conclude that HIV-1 mRNA is expressed by epidermal LC rather than by contaminating T cells. The finding that HIV-1 genes are expressed in LC from infected patients confirms that LC are productively infected *in vivo*.

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CYTOKINE- AND LPS-INDUCED CAT ACTIVATION IN MACROPHAGES AND LANGERHANS CELLS FROM TRANSGENIC MICE CARRYING THE LTR-CAT SEQUENCE OF HIV.
A.H. Warfel', G.J. Thorbecke', and D.V. Belsito''. Depts. of Dermatology' and Pathology',, NYU Med. Ctr., New York, NY 10016.
Infection by HIV-1 is characterized clinically by a long latency period preceding the development of AIDS. Both macrophage (MΦ) and Langerhans cell (LC) populations may exist that harbor integrated but unexpressed HIV provirus. Identification of factors that induce viral replication in these cells is important. Peritoneal MΦ from transgenic mice, carrying the HIV-LTR-CAT construct, were harvested and incubated for 48 h without or with various potential activators. The level of CAT activity was determined enzymatically. MΦs treated with either 250 U rmuIFN-γ/ml or 10 ng LPS/ml did not show much change in CAT activity, while cells incubated with both IFN-γ and LPS showed a 10-fold increase in CAT levels. The synergistic effect was LPS dose-dependent, but was still detected at 1 ng/ml. MΦs primed overnight with IFN-γ, washed, and then incubated with 10 ng LPS also showed increased CAT activity, whereas cells first incubated with LPS, followed by IFN-γ, did not. This synergistic effect is partly mediated by LPS-induced cytokines; both IL-6 and TNF-α act synergistically with IFN-γ. Preliminary data indicate a similar regulation of LTR in LC. However, studies on LC have been complicated by the expression of LTR-CAT in keratinocytes. These results suggest that cytokines and/or LPS act synergistically to induce HIV replication in MΦs and LC, and that IFN-γ plays a pivotal role. The relative importance of these factors in vivo is being evaluated in this transgenic mouse model. The priming effect of IFN-γ on cytokine-induced LTR expression may play a role in progression of AIDS in patients infected with HIV given reports of chronically elevated levels of cytokines, including IL-6, in this population.

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HUMAN PAPILLOMAVIRUS TYPE 16 VIRUS-LIKE PARTICLES: ELISA TO DETECT HIGH RISK HPV INFECTION AND POTENTIAL FOR A PREVENTIVE VACCINE. Reinhard Kirnbauer, Cosette Wheeler, Nancy Hubbert, Janet Taub, Richard Roden, Doug Lowy, John Schiller, Laboratory of Cellular Oncology, National Institutes of Health, Bethesda MD and Department of Cell Biology, University of New Mexico, Albuquerque NM.

Genital infection with "high risk" human papillomavirus (HPV), most often HPV16, is the most significant risk factor for the development of cervical cancer. To have a source of properly folded capsid proteins that might be used for developing a serological assay for HPV infection and for testing as a vaccine to prevent HPV infection, we have employed insect cells to produce the major capsid protein (L1) of HPV16. When the HPV16-L1 gene derived from a condyloma was inserted into a baculovirus vector and used to infect insect cells, preparative amounts of self-assembled HPV16-L1 particles that were indistinguishable from HPV virions were obtained. In contrast, L1 protein from the widely used HPV16 prototype strain, which we find contains a single amino acid change, did not assemble efficiently. Using the particles from the L1 that assembles efficiently, we have developed an ELISA and examined sera of 122 women with known HPV status. Two-thirds of woman positive for HPV16 DNA were positive in the ELISA, whereas 7% of HPV DNA negative women and 9% of (low risk) HPV6/11 positive women were ELISA positive. 82% of HPV16 positive women with cervical atypia or mild dysplasia were positive. Only 53% of women positive for HPV16 by PCR but with normal PAP smears were positive. These results, which require L1 particles because the serum antibodies recognize conformation dependent epitopes, suggest that it may be possible to develop an ELISA to detect clinically significant "high-risk" HPV infection. Since analogous L1 particles from bovine papillomavirus induce high titer neutralizing antibodies, the HPV16 L1 particles might have potential as a vaccine to prevent HPV infection.

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REGULATION OF MHC CLASS I, CLASS II AND ICAM-1 EXPRESSION BY TNFalpha AND RETINOIDS IN HPV16-HARBORING KERATINOCYTES Slawomir Majewski[1], Jacek Malejczyk[2], Francoise Breitburd[3], Gerard Orth[3] and Stefania Jablonska[1], Dept. of Dermatology[1] and Histology[2], Warsaw School of Medicine, Warsaw, Poland; Unite des Papillomavirus[3], Institut Pasteur, Paris, France

Expression of MHC class I, II and cellular adhesion molecules is a basic mechanism of immunosurveillance against tumor cells. Using quantitative ELISA and Northern blot we studied molecular mechanisms of MHC and adhesion molecules' expression in HPV16-harboring keratinocytes (Skv cells). Nontumorigenic Skv-e1 cells expressed high amounts of ICAM-1 and HLA-A,B,C proteins and mRNAs, as compared to Skv-e2 cells (tumorigenic counterpart). No significant HLA-DRα and LFA-3 molecule expression was detected in both Skv lines. ICAM-1, MHC class I and II expressions were upregulated by IFNγ, TNFα and retinoids (all trans retinoic acid, acitretin) and β-carotene, and this was not associated with changes in c-myc expression. ICAM-1 expression (but not MHC class I) was stimulated by TNFα-neutralizing antibodies, suggesting its autocrine induction by TNFα. Both tumorigenic and non-tumorigenic Skv cell lines released similar amount of TNFα; expression of TNFα receptor was, however, significantly increased in nontumorigenic Skv-e1 cells, as evaluated by ^{125}I-TNFα binding and mRNA analysis. The results suggest that differences in expression of adhesion molecules depend on tumorigenicity of HPV16-harboring cells. This could condition tumor cell susceptibility to the immune effector cells.

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THE RETINOBLASTOMA TUMOR SUPPRESSOR GENE IS A POTENTIAL TARGET OF INTERFERONS IN HPV INFECTED CELLS SK Tyring, I Arany and P Rady, Departments of Microbiology and Dermatology, University of Texas Medical Branch, Galveston, Texas, U.S.A.

Increases in TGF-beta 1, IFN-beta and RB mRNA levels, but a significant decrease in cdc2 kinase expression were found in condylomas or in oncogenic HPV-containing cell lines after in vivo or in vitro interferon (IFN) treatment. Immunodetection by Western blot demonstrated a higher proportion of unphosphorylated pRB in those treated cells, compared to the non-treated counterparts. The inhibitory effects of IFNs were further evidenced by decreased c-myc levels. However, decreased c-myc expression was significantly less in "high risk" HPV containing cells, than in "low risk" HPV specimens after IFN treatment. High levels of HPV 16 E7 which remained after IFN treatment, however, could abrogate the inhibitory effect of IFNs on c-myc expression by affecting the formation of the pRB/E2F complex which is responsible for that inhibition.

These results suggest that IFNs both in vivo and in vitro could interact with the RB tumor suppressor gene through an inhibitory cytokine pathway exerting their antiviral/antiproliferative effects on those HPV infected cells, but the "high risk" HPVs could abrogate this inhibitory effect via their E7 oncoproteins.

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TRANSFORMING CAPACITY OF CUTANEOUS HPV TYPE 5 AND TYPE 8 E7 GENES: COLLABORATION WITH *Rb* PROTEIN. Takeshi Nishikawa[1,2], Toshiharu Yamashita[2], Kaoru Segawa[3], Kei Fujinaga[2], Hitoshi Kobayashi[1] and Akira Ohkawara[1], [1]Department of Dermatology, Hokkaido University School of Medicine, Sapporo, [2]Department of Molecular Biology, Cancer Research Institute, Sapporo Medical University, Sapporo, [3]Department of Microbiology, Keio University, Tokyo, Japan.

Previously, we reported the collaborative transformation of primary rat embryo fibroblasts by HPV8 E7 and activated-H-*ras* (EJ-*ras*) gene (Jpn J Cancer Res 82:1340, 1991). In recent years, some roles of anti-oncogene (*Rb*) in the transformation induced by adenovirus, SV40 and HPV16 have been reported, as oncoproteins of adenovirus E1A, SV40 LT and HPV16E7 bind to *Rb* gene product to suppress the function of *Rb*. In order further to investigate the tumorigenicity of HPV5 and HPV8, the relation between transforming activity and *Rb* binding capacity was examined.

The E7 open reading frames of HPV5 and HPV8 as well as genital high risk HPV16 were cloned into the expression vector under the SV40 enhancer/promoter, and were transfected into primary baby rat kidney cells (BRK) by the calcium phosphate coprecipitation method, and the number of transformed colonies were counted. *Rb* binding capacity of HPV5, HPV8 and HPV16 E7 products was examined by immunoprecipitation method.

The transforming efficiency of the HPV5 and HPV8 E7 genes in BRK was lower than that of HPV16 E7 (HPV16 : HPV5 : HPV8 = 20 : 5 : 1). And, the *in vitro* binding activity of the *Rb* protein to HPV5 and HPV8E7 products was lower than that of the HPV16E7 product (HPV16 : HPV5 : HPV8 = 16 : 4 : 1). Correlation between transforming activity and *Rb* binding capacity indicates that Rb binding plays a key role in transformation by HPV.

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THE SUPRABASAL EXPRESSION OF $\alpha6\beta4$ INTEGRIN PREDICTS A HIGH RISK FOR MALIGNANT PROGRESSION IN MOUSE SKIN CARCINOGENESIS. Tamar Tennenbaum, Arin K. Weiner, Adam J. Belanger, Adam B. Glick, Henry Hennings and Stuart H. Yuspa, Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, Bethesda, MD, USA

Enhanced expression of the $\alpha6\beta4$ integrin complex has been linked to malignant progression in mouse skin carcinogenesis. To determine if $\alpha6\beta4$ expression can predict risk for malignant conversion among populations of benign skin tumors, we analyzed the distribution of $\alpha6\beta4$ and other markers of progession in papillomas at high and low risk for malignant progression. After initiation with 7,12-dimethylbenz[a]anthracene, mice were promoted with 12-O-tetradecanoylphorbol-13-acetate to induce predominantly low risk tumors or promoted with mezerein to produce predominantly high risk tumors. When tumors first appeared at 8 weeks after promotion, high risk papillomas demonstrated basal and suprabasal $\alpha6\beta4$ expression, loss of keratin 1 and aberrant expression of keratin 13. In these tumors $\alpha6\beta4$ expression coincided with an expansion of the proliferating compartment as indicated by suprabasal bromodeoxyuridine labeling. In contrast, $\alpha6\beta4$ immunostaining was confined to basal cells in low risk tumors, keratin 1 was abundant and keratin 13 was absent in the majority of this group, while proliferating cells were largely in the basal compartment. By 33 weeks, $\alpha6\beta4$ suprabasal expression continued to distinguish groups at higher risk for malignant conversion, but keratin 13 was expressed in all groups. At this time, high risk tumors displayed focal expression of keratin 8 and γ-glutamyltranspeptidase, markers also found in chemically induced carcinomas. Keratin 8 and γ-glutamyltranspeptidase were only expressed in $\alpha6\beta4$ positive cells. These results indicate that expression of $\alpha6\beta4$ integrin in suprabasal strata serves as an early predictive marker to identify benign squamous tumors at high risk for malignant progression.

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PROTECTION AGAINST TUMOR PROMOTER-INDUCED INFLAMMATION IN MURINE SKIN BY GREEN TEA POLYPHENOLS. Hasan Mukhtar, Santosh K. Katiyar and Rajesh Agarwal, Department of Dermatology, Case Western Reserve University, Cleveland, Ohio, USA

In prior studies we and others have shown that oral feeding or topical application of a polyphenolic fraction isolated from green tea (GTP) affords protection against 12-O-tetradecanoylphorbol-13-acetate (TPA)- and ultraviolet B (UVB) radiation-induced tumor promotion in murine skin. It is known that exposure of murine skin to TPA or UVB radiation results in cutaneous edema, epidermal hyperplasia, depletion of antioxidant-defense system and induction of epidermal ornithine decarboxylase (ODC) and cyclooxygenase activities. In order to assess the protective effects of GTP against inflammation, in this study we evaluated the protective effect of GTP on these TPA- and UVB radiation-caused changes in murine skin. Topical application or oral feeding of GTP prior to exposure with TPA or UVB resulted in significant protection against these two tumor promoter-caused cutaneous edema ($p < 0.0005$), epidermal hyperplasia (p , 0.0001) and depletion of antioxidant-defense system in epidermis ($p < 0.01$ to 0.02). Pre-treatment with GTP also resulted in significant protection against TPA- and UVB radiation-caused induction of epidermal ODC ($p < 0.005$ to 0.01) and cyclooxygenase activities ($p <$ 0.0001) in a time-dependent manner. Our data indicate that the inhibition of TPA- and UVB radiation-caused changes in these markers of tumor promotion in murine skin by GTP may be one of the possible mechanisms of chemopreventive effects associated with green tea against tumorigenesis. The results of this study suggest that green tea, specifically polyphenols present therein, may be useful against inflammatory responses associated with the exposure of skin to chemical tumor promoters as well as to solar radiations.

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RAS p21 FARNESYLATION IN ULTRAVIOLET RADIATION-INDUCED TUMORS IN MURINE SKIN. R. Agarwal, S.G. Khan and H. Mukhtar, Department of Dermatology, Case Western Reserve Univ., Cleveland, Ohio, USA

Cutaneous exposure to solar ultraviolet B (UVB) radiation is well recognized as the major cause of skin cancer in humans, however, the precise molecular mechanisms whereby UVB mediates carcinogenesis remains elusive. The involvement of ras oncogenes *viz.* Ha-, Ki-, and N-ras has been extensively studied in animal and human cancers. These genes encode for structurally related 21 kDa proteins (ras p21) that are involved in the transduction of extracellular signals controlling cell growth. The activated ras genes encode mutated p21 that exist in the GTP-bound active state, and following its localization to the inner side of the plasma membrane, cause cellular transformation by stimulating uncontrolled tumor growth. This membrane association requires three post-translational modifications occurring at the C-terminus of the ras p21. The farnesylation of p21 by a cytosolic enzyme farnesyltransferase (FTase) is the key step that triggers biological functions of p21, and promotes the interaction of ras oncoproteins with effector molecules necessary for transformation. In this study, FTase activity was found to be substantially higher (~3-fold) in UVB radiation-induced tumors in SKH-1 hairless mice compared to unirradiated normal epidermis. Western blot analysis employing anti-Ha-ras Ab-1 showed higher levels of Ha-ras p21 in both cytosolic and membrane fractions prepared from tumors compared to epidermis. Pan ras antibody against mutated p21 at codon 12 showed the presence of ras val-12 p21 in UVB-induced tumors but not in normal epidermis, suggesting a gly to val mutation at codon 12 in ras p21 in UVB radiation-induced cutaneous neoplasms in SKH-1 hairless mice. Our data indicate that enhanced FTase activity and the processing of overexpressed p21 in UVB-induced tumors are correlated, and predict the role of point mutations at the 12th codon of the ras oncogene during photocarcinogenesis in mice.

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UV HYPERMUTABILITY OF A SHUTTLE VECTOR: PROGRESS TOWARD AN ASSAY FOR DYSPLASTIC NEVUS SYNDROME. Shin-Ichi Moriwaki and Kenneth H. Kraemer, National Cancer Institute, NIH, Bethesda, MD, USA.

The diagnosis of some forms of the melanoma prone disorder, dysplastic nevus syndrome (DNS) currently is based on a disputed combination of clinical and histopathological features of patients. In order to develop a laboratory test for DNS, we are using a UV-treated shuttle vector plasmid which has an abnormally increased frequency of mutations after repair in lymphoblastoid cells from a patient with DNS (Cancer Res 49: 5918, 1989) or with xeroderma pigmentosum (XP). The plasmid, pSP189 (with a gene for ampicillin resistance) is treated with UV and transfected into human lymphoblastoid cells. Untreated pZ189K (with a gene for kanamycin resistance) is co-transfected as an internal standard. After replication and repair in the human cells for 2 days, plasmids are extracted and used to transform an indicator strain of E. coli with an amber mutation in the lacZ gene. The ratio of amp^R bacterial colonies to kan^R colonies is a measure of plasmid survival. The proportion of light blue or white colonies (representing inactivating mutations in the supF marker gene in the plasmid) is a measure of the mutation frequency. Survival of UV treated plasmid was reduced after passage through XP-A and XP-G cells but was normal in 3 familial DNS lines. The frequency of UV-induced mutations in plasmids recovered from XP-A and XP-G cells was 3-6 fold greater than with the normal. Transfection of UV treated plasmid by DEAE dextran (but not by electroporation) yielded 3-4 fold increased mutation frequency with 3 familial DNS lines. These results suggest that plasmid UV-hypermutability with normal UV survival may form the basis of a laboratory assay for DNS.

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PAPULONODULAR AND MORPHEAFORM BASAL CELL CARCINOMAS (BCC) DISPLAY EXTENSIVE ABNORMALITIES IN EPIDERMAL BASEMENT MEMBRANE COMPONENTS. NJ Korman, SL Hrabovsky. Case Western Reserve University, Cleveland , OH USA.

BCC is the most common human cancer. While the etiologic role of sunlight in the development of BCC is established, the factors which control this tumors behavior are not well understood. We studied the basement membrane (BM) biology of BCC by indirect immunofluorescence to determine whether alterations in BM components may play a role in BCC tumor invasion. We studied a total of 16 papulonodular BCC's (PNBCC) and 3 morpheaform BCC's (MBCC). The 230 KD bullous pemphigoid antigen (BPA) was either not detected (13/16) or significantly diminished (3/16) in PNBCC tumor BM while the 180 KD BPA was uniformly undetectable in PNBCC tumor BM (16/16). Epiligrin was not detected (9/15) or minimally expressed (6/15) in PNBCC tumor BM. α_6 integrin was not detected (15/16) or minimally expressed (1/16) in PNBCC tumor BM, and β_4 integrin was uniformly undetectable in PNBCC tumor BM (16/16). Type VII collagen was not detected (9/16) or significantly decreased (4/16) in PNBCC tumor BM. Laminin and type IV collagen expression were both at least as strong in PNBCC tumor BM as in adjacent normal BM. All three MBCC's displayed undetectable levels of the 230 and 180 KD BPA's, the α_6 and β_4 integrin subunits and epiligrin and diminished levels of both laminin and type IV collagen. All BM components were present both in the epidermis of normal skin as well as in the epidermal BM overlying BCC tumor nests. Our findings reveal extensive defects in numerous components of the BM of BCC's with even more significant abnormalities found in MBCC's than in PNBCC's. These findings suggest that these major BM abnormalities may facilitate or contribute to BCC tumor invasion. Studies are underway to determine the mechanism underlying these abnormalities.

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EXPRESSION OF TYROSINASE, TYROSINASE-RELATED PROTEIN 1 AND 2, C-KIT AND C-KIT LIGAND IN VITRO AND IN VIVO. Chie Sakai and Koichiro Kameyama, Department of Dermatology, The Kitasato Institute Medical Center Hospital, Saitama, Japan

Expression of tyrosinase and tyrosinase related proteins 1 and 2 (TRP1 and TRP2,DOPAchrome tautomerase) were studied in vitro using highly pigmented and amelanotic human melanoma cell lines and in vivo using normal skin and hyperpigmented disorders. The results showed that there was a positive correlation between quantities and synthetic rates of those melanogenic enzymes and their melanin formation and DOPAchrome tautomerase activities. The effect of a heat resistant melanogenic inhibitory factor on the oxidation of 5,6-dihydroxyindole (DHI) was also studied, and showed that the inhibitory factor significantly suppressed the oxidation of DHI with or without tyrosinase. Immunohistochemistry showed that melanocytes at the basal layer of normal skin were tyrosinase negative,TRP1 negative, TRP2 positive, c-kit positive but Steel factor negative. However, melanocytes of patients with hyperpigmentary disorders became tyrosinase, TRP1 and TRP2 positive. Surprisingly, dendritic or spindle shaped cells, which were positive for all melanogenic enzymes, were observed not only in the skin of 7 cases of acquired dermal melanocytosis but also in all 19 cases of normal skin. These results clearly suggest that all three melanogenic enzymes as well as c-kit play an important role on the regulation of melanin production, and that melanoblasts exist in the dermis of normal skin.

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ROLE OF COATED VESICLES AND PREMELANOSOMES IN THE FORMATION OF MELANIN POLYMER FROM ITS MONOMERS. Adam Wilczek and Yutaka Mishima, Mishima Institute for Dermatological Research, Kobe, Japan

5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHI2C) are the main monomers of eumelanin. Both can polymerize spontaneously *in vitro* systems, but the process of polymerization of DHI2C proceeds far more slowly under physiological pH. Therefore, substantial incorporation of DHI2C into melanin polymer synthesized within living pigment cells raises the question whether this process is up-regulated by some factors *in vivo*. We have studied the mechanisms regulating formation of DHI2C-melanin polymer within various melanogenic subcompartments in melanoma cells. The coated vesicle (CV) and premelanosome (PMS) fractions have been isolated from Greene's melanoma by sucrose density gradient ultracentrifugation, and their role as distinctive subcompartments for polymerization of DHI2C has been analyzed by HPLC. We have found that both melanogenic compartments accelerate the formation of melanin polymer from DHI2C *in vitro*. This activity adheres to Concanavalin-A (Con A) and can be inhibited by phenylthiourea (PTU) - a potent tyrosinase inhibitor. However, in Con A affinity chromatography, the elution profile of tyrosinase differs from that of DHI2C-polymerizing factor. We suggest that the formation of DHI2C-melanin polymer is up-regulated *in vivo* by a functioning glycoprotein different from tyrosinase.

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TYROSINASE cDNA TRANSFECTION INDUCES CHAIN REACTIONS NECESSARY FOR MELANIN-POLYMER FORMATION IN FIBROBLASTS. H. Kondoh[1], S. Narimizu[1], O. Ando[3], and Y. Mishima[1,2]. [1]Mishima Institute for Dermatological Research, Kobe; [2]Kobe Kaisei Hospital, Kobe; [3]Hayashibara Biochemical Laboratories Inc., Okayama, Japan.

Melanin-polymer formation needs not only the tyrosinase (Ty-ase) biosynthesis, but also its glycosylation, premelanosome (PMS) formation, Ty-ase transportation to PMS by coated vesicles (CV), and many other factors within the pigment cell. Furthermore, the late polymerization process needs the enzymatic activities of dopachrome tautomerase, DHI2C-oxidase, and so on. Genes of these enzymes have been cloned.

To introduce one of these regulatory factors, we transfected Ty-ase cDNA into mouse L929 fibroblasts.

Electron microscopy of pigmented transformant, LHT2, exhibited the Ty-ase maturation and transporting process in GERL-CV system in the same way as in pigment cells. Melanin-polymer appeared in cytoplasmic vacuoles which showed positive PMS reaction (Y. Mishima, *J.I.D.*, 1962) and a stepwise melanization process similar to melanosome stage I~IV. Further, these organelles showed acid phosphatase activity and Latex particle phagocytosis common to lysosomes.

These results seem to indicate that Ty-ase cDNA transfection alone is sufficient to trigger or induce the multiple chain reactions necessary for melanin-polymer formation in fibroblasts, without transfection of any other genes which encode regulatory factors.

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DYNAMICS OF DOPACHROME TAUTOMERASE AND PROTEIN KINASE C ACTIVITIES IN MELANOGENIC SUB-CELLULAR COMPARTMENTS FOLLOWING TYROSINASE-cDNA TRANSFECTION. Takakazu Shibata and Yutaka Mishima, Department of Dermatology, Kobe Kaisei Hospital and Mishima Institute for Dermatological Research, Kobe, Japan

Tyrosinase-related protein-2(TRP-2) has been recently identified as dopachrome tautomerase(DT) which plays a critical role in the post-tyrosinase melanin polymer forming pathway. In 1987 we reported that melanin monomers, dihydroxyindole(DHI), and dihydroxyindole-2-carboxylic acid(DHI2C) which is formed from dopachrome by DT, were already rich in coated vesicles(CV) which transport tyrosinase into premelanosomes(PMS).

Using sub-cellular fractionation methods,we have found that DT activity is 20 times higher in CV than in PMS. We have further investigated using its specific antibody by immuno-electron microscopic technique,the distribution of DT in various melanogenic sub-compartments of B16 cells. The dynamics of tyrosinase and DT activity were investigated,not only in amelanotic cells but also in tyrosinase cDNA transfection induced melanotic cells. CV,especially larger ones,exhibited anti-DT antibody deposits.

When PKC-enhancer or inhibitor was added into cultured B16 melanoma cell system, melanin synthesizing activity decreased or increased. We also investigated the intra-cellular localization and amplification of PKC activity in variously pigmented melanoma cells using its specific antibody by immuno-electron microscopic technique. We have found that PKCα-subspecies' activity was distinctly amplified and localized at cell membrane and peri-nuclear area and the activity in tyrosinase cDNA transfection induced melanotic cells decreased compared with its original amelanotic cells.

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SIGNAL TRANSDUCTION THROUGH PROTEIN KINASE C SUBSPECIES IN THE MITOGENESIS OF NORMAL HUMAN MELANOCYTES. Masahiro Oka, Hideya Ando, Tatsuya Horikawa, Kazuhito Hayashibe and Masamitsu Ichihashi, Department of Dermatology, Kobe University School of Medicine, Kobe, Japan

Normal human melanocytes can grow in the presence of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) or basic fibroblast growth factor (bFGF) *in vitro*. It is generally accepted that the major intracellular receptor for TPA is protein kinase C (PKC). Molecular cloning and biochemical analysis have revealed the existence of multiple subspecies of PKC, and at the last count, ten subspecies (α, βI, βII, γ, δ, ε, η, θ, ζ, and λ) of PKC have been identified in mammalian tissues. TPA directly activates PKC in cells and also elicits degradation, termed "down-regulation", of PKC. bFGF also induces activation of PKC through phosphatidylinositol hydrolysis by activation of phospholipase Cγ. To elucidate the physiolosical role of PKC for the proliferation of human melanocytes, expression and activities of PKC subspecies in TPA-dependent and bFGF-dependent human melanocytes were examined, respectively. Biochemical and immunoblot analyses have revealed that the α-, β-, δ-, ε-, and ζ-subspecies of PKC are expressed in bFGF-dependent melanocytes, whereas PKC is almost depleted except for the ζ-subspecies in TPA-dependent cells. These results indicate that normal human melanocytes show different expression of PKC subspecies according to growth stimulants and that down-regulation of PKC is not essential for the growth of these cells *in vitro*. Furthermore, it is suggested that the ζ-subsecies of PKC plays a role in the mitogenic signalling.

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REGULATION OF MELANOGENESIS BY PROTEIN KINASE C (PKC) BETA. Hee-Young Park, Patricia Roddey, Masahiro Hara, Vladimir Russakovsky, Esther Fernandez and Barbara A. Gilchrest, Department of Dermatology, Boston University School of Medicine, Boston, MA

Extensive work over at least five decades has identified the cell, organelle, and rate limiting enzyme involved in melanogenesis, but the signal transduction pathway for melanogenic signals remained poorly understood. We found that addition of the endogenous activator of PKC, diacylglycerol, to cultured human melanocytes (Mc) increases melanin content; that tyrosinase (T) activity parallels total PKC activity and specifically the level of PKC-β isoform mRNA and protein. To determine if PKC-β is required for melanogenesis, we used a non-pigmented human melanoma line (NP-MM4) that does not express PKC-β and has undetectable T activity, as well as the pigmented parental MM4 line that expresses PKC-β and has comparable T protein level and growth rates. When PKC-β was introduced to NP-MM4 cells, either by transfection or by mixing together cell lysates from NP-MM4 cells lacking PKC-β and human Mc expressing PKC-β, T was activated. The mechanism of PKC β's melanogenic effect was elucidated by activating PKC with phorbol ester in the presence of (ortho-^{32}P) ATP then immunoprecipitating T using a polyclonal antibody. A single band of ^{32}P was identified at 70 KD, the known size of T, suggesting activation of T via phosphorylation by PKC. Immunoelectron microscopy revealed co-localization of T and PKC-β proteins at the melanosome membrane in cultured Mc. Furthermore, in Cloudman S91 cells, depletion of PKC blocked alpha-melanocyte stimulating hormone (MSH)-induced pigmentation and induction of T protein. Conversely, MSH-induced pigmentation was associated with increases in PKC-β message and protein. Taken together, these data establish a key role for PKC-β in both human and murine melanogenesis.

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MODULATION OF MELANOCYTE RESPONSES BY CALCIUM. P.S.Friedmann, C.J.Carsberg, K.T.Jones and G.R.Sharpe, Department of Dermatology, University of Liverpool, U.K.

Ultraviolet radiation (UVR) stimulates melanocytes (MCs) to produce melanin pigment, which is transferred via dendrites to surrounding keratinocytes (KCs). The mechanisms underlying these events are unknown. Since calcium is a modulator of many cellular responses, we investigated the effects of raising the extracellular calcium concentration ($Ca^{++}o$) on both the baseline melanin content of human MCs and also their responses to UVR and other melanogenic stimuli. In addition, because the intercellular contacts may be important in regulation, the intracellular free calcium concentration ($Ca^{++}i$) was determined in MCs in the presence or absence of contact with KCs.

For melanogenic responses MCs were cultured in MCDB153 (70μM $Ca^{++}o$) with 100μg/ml bovine hypothalamic extract, 1nM cholera toxin and 3% FCS. For $Ca^{++}i$ measurement mixed cultures of MCs and KCs were grown in MCDB153 and loaded with the calcium-sensitive dye fura-2.

Raising $Ca^{++}o$ to 1.8mM for 3 days caused an increase in melanin content to 210% of control and increased sensitivity to melanogenic stimuli (Table). Results are from 3 replicate experiments.

Table	70μM	1.8mM $Ca^{++}o$
Control	100*	210
UVR	174	403
OAG (60μM)	834	1874
Staurosporine (10nM)	208	419

* Melanin/cell (% control in 70μM $Ca^{++}o$)

MCs grown in 70μM $Ca^{++}o$ medium had a resting $Ca^{++}i$ of 90±3nM (mean±SEM, 120 individual cells). Raising $Ca^{++}o$ to 1.8mM for 24h resulted in a rise in $Ca^{++}i$ to 151±6nM ($p<0.001$, Mann Whitney U test). There was no significant difference between MCs with or without KC contacts.

In MCs increasing the $Ca^{++}o$ leads to an elevated $Ca^{++}i$, which is accompanied by increased baseline melanin content and also by augmented sensitivity to melanogenic stimuli. These results support a role for calcium as a modulator of melanogenesis in MCs.

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THE ROLE OF CATECHOLAMINES IN VITILIGO. Karin U. Schallreuter[1], K. Regina Lemke[1], John M. Wood[1], Wiete Westerhof[2] and Anthony J. Thody[3], Department of Dermatology, University of Hamburg, Germany. Department of Dermatology, Academic Medical Center Amsterdam, The Netherlands, [3]Department of Dermatology, University of Newcastle-upon-Tyne, England

Recent results indicate that vitiligo is primarily a disorder of keratinocytes leading to an instability of the delicate symbiotic relationship between the 36 keratinocytes to one melanocyte in the epidermal unit. It has been shown that a defect in calcium transport occurs in keratinocytes established from lesional skin in vitiligo, and that this defect is promulgated by abnormalities in both the biosynthesis of catecholamines and the expression of the beta-2-adrenoceptor signal transduction system, a pathway which regulates the intracellular calcium levels. Vitiliginous keratinocytes present an increased density of beta-2-adrenoceptors on differentiating cells correlating with a defective $^{45}Ca^{++}$ uptake compared to non-lesional and control keratinocytes. Biopterin-dependent tyrosine hydroxylase (TH) activity was significantly higher in vitiliginous epidermal cell extracts using ^{3}H exchange from 3,5 ^{3}H ring labeled L-tyrosine and by immunohistochemistry with a monoclonal antibody against TH. Also, keratinocytes grown in vitro showed biopterin-dependent control of beta-2-adrenoceptor density. Phenylethanolamine-N-methyl-transferase activity was significantly lower in vitiliginous epidermis (n•14). As a consequence of these changes in the catecholamine biosynthetic pathway, norepinephrine builds up yielding (a) increased urinary excretion of norepinephrine in 31% of vitiligo cases examined (n•90)(this correlates with higher levels of catechol-O-methyl transferase (COMT) metabolites), (b) an increased COMT activity in vitiliginous epidermis>perilesional skin>non-lesional skin>controls and (c) an increased norepinephrine content in perilesional melanocytes. We have shown that calcium regulates the biosynthesis of the essential cofactor tetrahydrobiopterin, the electron donor for TH, and that this cofactor represents the fluorescence emission at 359 nm shown by Wood-light in the diagnosis of vitiligo. Taken together, our results and the results from other groups suggest that vitiligo may be primarily a defect in biopterin production leading to an unregulated catecholamin biosynthesis in the epidermis of these patients.

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MOLECULAR CHARACTERIZATION AND TISSUE DISTRIBUTION OF NOVEL, IMMUNOGENIC MELANOMA-ASSOCIATED ANTIGENS SYTHESIZED IN VITRO. Kazuhito Hayashibe, Hidekazu Tsukamoto, Satoru Kako, Hiroshi Nagai, Masamitsu Ichihashi and Soldano Ferrone, Department of Dermatology, Kobe University School of Medicine, Kobe, Japan and Department of Microbiology and Immunology, New York Medical College, NY, U.S.A.

Identification and characterization of human melanoma-associated antigens immunogenic in patients with melanoma is one of the most critical challenges to understand the immunological events during the course of the disease and to develop immunogens useful to implement active specific immunotherapy in patients with melanoma. We recently reported the identification of novel melanoma-associated antigens immunogenic in patients by screening an expression cDNA library constructed with a cultured human melanoma cell line mRNA utilizing sera from patients with melanoma. In-vivo expression and distribution of the cloned gene (1029bp) and its coding peptide have been examined by in-situ hybridization with riboprobe and immunostaining with polyclonal anti-peptide antibodies raised in mice. Positive hybridization was observed heterogeneously in the cytoplasm of melanoma cells, along with the positive reactivities of polyclonal anti-peptide antibodies, which were rather restricted in melanoma cells except some cuboidal cells of stomach wall closely adjacent to metastatic melanoma cells. Nucleic acid sequence of the above cDNA (1029bp) has approximately 30% homology with the other clone in process of characterization , indicating the code for different antigens. These information will assess the suitability of recombinant peptide antigen to implement active specific immunotherapy in patients with melanoma.

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MELANOGENESIS-SEEKING ^{18}F-^{10}B-L-DOPA ANALOGUE FOR SELECTIVE DETECTION AND ERADICATION OF HUMAN MALIGNANT MELANOMA.

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The selective *in situ* detection and eradication of cancer using its specific metabolic activity has long been a challenge. Our idea exploits the accentuated melanogenesis within melanoma cells to selectively destroy them.

The synthesis of ^{10}B-*p*-borono-phenylalanine (^{10}B-L-BPA) having the ability to accumulate within melanosomes, was developed by us for use in Neutron Capture Therapy (NCT). In NCT the administration of ^{10}B-L-BPA is coupled with relatively harmless thermal neutron irradiation, releasing charged α-particles and ^{7}Li atoms. These high LET particles travel a distance of 14μm from the activated ^{10}B atoms, which is approximately the diameter of individual melanoma cells, thus selectively eradicating them.

After basic investigation and successful treatment of 10 cases of human primary melanoma, we have succeeded in treating deep-seated human metastatic melanoma by NCT after introducing significant replacement of tissue H_2O with D_2O by oral administration of heavy water, resulting in higher neutron penetration.

Melanoma specific affinity of ^{10}B-L-BPA, confirmed by NCT clinical data, has led us to develop a novel specific *in situ* detection method for melanoma. This has been accomplished by further synthesizing ^{18}F-^{10}B-L-BPA, on site as the half life of ^{18}F is only 109 min., for the application of Positron Emission Tomography (PET) which currently uses non-specific compounds such as ^{18}F-fluoro-deoxyglucose.

It has been found that PET with ^{18}F-^{10}B-L-BPA provides not only 3-dimensional imaging specific for melanoma, but also *in situ* determination of ^{10}B concentration in the target cancer which offers crucial information for dose planning not only for NCT but also for the development of other therapeutic modalities.

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CLINICAL RELEVANCE OF ICAM-1 EXPRESSION IN PRIMARY LESIONS AND SERUM OF PATIENTS WITH MALIGNANT MELANOMA.

Toshiro Kageshita and Tomomichi Ono, Department of Dermatology, Kumamoto University School of Medicine, Kumamoto, Japan.

ICAM-1 is a sialylated glycoprotein which binds to the leukocyte integrins LFA-1 and Mac-1 to support cell-cell adhesion and induction and effector functions in the immune response. The potential clinical relevance of the level of ICAM-1 prompted us to measure ICAM-1 in primary lesions and in serum of melanoma patients and correlate these parameters with the clinical course of the disease. Immunohistochemical staining revealed that ICAM-1 was expressed in about 70% of primary lesions and 90% of metastatic lesions. ICAM-1 expression in primary lesions was significantly associated with their thickness and a reduction in the disease free interval and survival. The mean serum ICAM-1 level in melanoma patients with stage 1 & 2 was not significantly different from that of healthy control, however the level of serum ICAM-1 was significantly increased in patients with stage 3 & 4. And the level of serum ICAM-1 in patients with liver metastasis was significantly higher than in thoes with other organ metastasis and related with disease progression. These results suggest that ICAM-1 expression in primary lesions is a prognostic marker and serum level of ICAM-1 may represent a useful approach to detect liver me tastasis and to monitor the disease progression.

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CLINICAL ACTIVITY OF A POLYVALENT MELANOMA VACCINE.

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We have developed a partially purified, melanoma vaccine from cell-surface material shed into culture medium by melanoma cells. The vaccine is polyvalent and contains a broad range of melanoma associated antigens. This analysis was conducted to examine the clinical activity of the vaccine.

We measured vaccine induced antimelanoma cellular and humoral immune responses and disease-free and overall survival in 94 pts with surgically resected stage III (regional disease) malignant melanoma. We found that both disease-free (DF) and overall survival (OS) of vaccine treated pts was 50% longer than that of similar historical controls. Survival was particularly prolonged in pts who developed an immune response to vaccine treatment. Vaccine treatment induced antimelanoma cellular immune response (measured by DTH response to skin tests) and/or antibodies (measured by specific immunoprecipitation) in over 50% of pts. Median DF survival was 4.7 yrs longer and OS 3.7 yrs longer in pts with a strong, as opposed to no, cellular immune response to the vaccine ($p = <0.02$). Similarly, median DF survival of pts with vaccine induced melanoma antibodies was significantly longer than that of non-responders (47 mo vs 19 mo respectively, $p=0.001$) as was overall survival (62 mo vs 46 mo, $p=0.01$). These differences in outcome were unrelated to disease severity or to the overall immunological competence of the pts, suggesting they resulted from vaccine treatment. Side effects developed in only one patient.

Thus melanoma vaccine treatment is safe, capable of stimulating antimelanoma immunity in many pts, and appears to be clinically effective in delaying the progression of this cancer in some individuals.

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ACTIVE SPECIFIC IMMUNOTHERAPY FOR MALIGNANT MELANOMA WITH ALLOGENEIC MELANOMA VACCINE: DYNAMICS OF IMMUNOLOGICAL PARAMETERS ALONG WITH CLINICAL RESPONSE

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Despite of several elaborate vaccination for patients with melanoma, the evaluation for active immunotherapy is still vague mainly due to the lack of information on monitoring immunological response of patients along with their course of disease. To provide a firmer foundation for active specific immunotherapy, we have analyzed immunological parameters on partial response (PR) patient compared to non-response (NR) patient received allogeneic melanoma vaccine. Preparation of allogeneic melanoma vaccine was carried out by sonication and treatment with mitomycin C. The vaccine-BCG mixture was injected intradermally every one month into the patients. The patients were pre-administrated by cyclophosphamide (300 mg / m^2, i.v.) and post-administrated by recombinant interleukin-2 ($1x10^6$ units, s.c.).

In immunohistological study of cutaneous metastatic lesions which showed distinct regression at 14 weeks, we have observed the enhanced expression of ICAM-1 and the dense infiltration of predominantly OKT4-positive lymphocytes at the deep portion of its nodule. The patient with PR showed the augumentation of delayed-type hypersensitivity to allogeneic melanoma cells, increase of the number of OKT8 in peripheral blood resulting in inversion of the OKT4 / T8 ratio, compared with patient with NR. The specific binding of serum from PR patient with allogeneic cultured melanoma cells was detected to be distinctly increased at 18 weeks after first vaccination. The above information will assess the suitability of continuous vaccination with allogeneic melanoma cells during the course of disease.

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DESMOGLEIN-1 CONTENT IN THE STRATUM CORNEUM IS DIRECTLY INVOLVED IN THEIR ADHESIVE PROPERTIES

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Aberration in cohesive components, such as stratum corneum lipid and desmosomal proteins, is associated with altered desquamation. However, the relationship between the magnitude of intercellular adhesion and quantitative changes in cohesive components remains obscure. In order to understand biochemical event in desquamatory process which leads to an alteration of adhesion in the stratum corneum, we quantified a desmosomal membrane core components, Desmoglein-1, in stratum corneum layer (SC layer) exfoliated from the surface of the skin by cyanoacrylate resin in relation to altered adhesive properties as seen in the normal and UVB-exposed skin. In the normal skin, Desmoglein-1 showed significant regional variation with the sole containing several times larger amount than the upper arm. On the other hand, the sole displayed the higher magnitude of cohesion between the stripped SC layer than the upper arm. In UVB-induced flaky skin, the number of the stripped SC layer increased in a UVB dose-dependent manner, with elevated Desmoglein-1 content per mg extracted proteins. These results indicate that changes in Desmoglein-1 content are associated with an altered intercellular adhesion in normal as well as UVB-exposed skin, suggesting that desmosomal proteins play a role in augmented adhesion as a cohesive component in the stratum corneum.

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PEMPHIGUS-IgG (P-IgG) BINDING TO CULTURED NORMAL AND CARCINOMA A-431 KERATINOCYTES ELICITS INTRACELLULAR Ca^{2+} ($[Ca^{2+}]in$) TRANSIENTS AND PROTEIN KINASE C (PK-C) MEMBRANE TRANSLOCATION

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The mechanism of P-IgG induced acantholysis is as yet unknown. We investigated whether changes in cellular signalling are involved in this process. High $[Ca^{2+}]$ (>0.1mM) grown cells binds P-IgG in bimodal pattern: high affinity sites (Kd≈0.2 mg/ml, saturation at 1 mg IgG/ml) and low affinity (LA) sites (Kd ≈ 1 mg/ml, saturation at 3-4 mg IgG/ml). Addition of P-IgG to Fura-2 loaded keratinocytes cause rapid (within 2-5 sec at 30°C) transient increase in $[Ca^{2+}]in$; from 50-100nM to 400-800nM, returning to basal level within 5-10 min as measured by image analysis of individual cells. The $[Ca^{2+}]$ transients were not seen with normal IgG and were dose-dependent on P-IgG LA sites saturation. While proliferating cells could respond secondarily to various agonist(s) (β-adrenergic etc.) by transient increase in $[Ca^{2+}]in$, differentiating cells responded only to P-IgG addition. Rapid PK-C membrane translocation ($t_{\frac{1}{2}}$ = 5 min), dose dependent on P-IgG binding to LA sites, was also found in cultured cells. Cell detachment and culture "acantholysis" at 60-70 hrs, were found when P-IgG was added to saturate the LA sites. Thus, P-IgG might elicit pathological changes in differentiated keratinocytes via membrane signalling system, initiating with transient increase in $[Ca^{2+}]in$, following P-IgG binding.

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A COMPREHENSIVE PROTEIN DATABASE APPROACH TO THE STUDY OF PSORIASIS: TOWARDS MOLECULAR DIAGNOSTICS.

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A great deal of research is currently being devoted to the study of psoriasis, a hyperproliferative disease that is accompanied by inflammation and infiltration of leukocytes. At present, relatively little is known about the molecular mechanisms underlying this disease, and in general, most studies have been directed towards the involvement of cytokines, growth factors and derivatives of arachidonic acid. Using a two-dimensional gel protein database approach, we have identified a group of low molecular weight proteins that are highly up-regulated in unfractionated non-cultured psoriatic keratinocytes and that may be related to the pathophysiology of the disease. These include: psoriasin; PA-FABP (psoriasis associated fatty acid binding protein); MRP 8; MRP 14; the interleukin-1 receptor antagonist; cystatin A; keratin 16 and several unknown proteins. Psoriasin and PA-FABP have been cloned by using oligodeoxyribonucleotides derived from backtranslated peptide microsequences obtained from two-dimensional gelelectrophoresis purified protein. We have also cloned a lectin-like protein that is moderately down-regulated in psoriatic keratinocytes. Psoriasin and PA-FABP are strongly upregulated at the transcriptional level in psoriatic keratinocytes. Two unknown proteins called phorbolin 1 and 2 that are upregulated in psoriatic keratinocytes, can be induced by PMA (12-myristate-13-acetate), suggesting that these proteins may be involved in the PKC pathway. Expression of most of these proteins can be induced in normal keratinocytes by addition fetal calf serum to medium and thereby - at least in part - resembling psoriatic keratinocytes.

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LOCATION AND CHARACTER OF KERATINOCYTE MUSCARINIC ACETYLCHOLINE RECEPTORS.

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We previously reported that human keratinocytes (HK) synthesize, store, secrete and degrade acetylcholine, and that HK express high density muscarinic acetylcholine receptors (mAChR) to mediate cholinergic regulation of their proliferation, differentiation, migration, cell-substrate and cell-cell attachments, and cytoplasm motility. We now visualized mAChR by anti-mAChR monoclonal antibody (MoAb) M35 (Chemunex, France) using indirect immunofluorescence, immunogold electron microscopy and immunoblotting. In human skin, MoAb M35 produced intercellular staining in basal, spinous and granular epidermal layers and labeled corneocytes in the stratum corneum. In HK monolayers, mAChR was found both expressed on the cell membranes and internalized by the cells. The exposure of cultured HK to muscarinic agonists and antagonists caused redistribution of the receptors. Increasing the concentration of extracellular Ca^{2+} from 0.09 to 1.6 mM upregulated mAChR expression and caused aggregation of the receptors in the sites of cell-cell contacts. The use of MoAb M35 to probe mAChR among HK proteins separated by SDS-PAGE or by isoelectric focusing revealed a core protein with approximate molecular weight of 60 kDa and pI about 4.0. The processing of mAChR by HK apparently yielded several smaller and less acidic proteins recognized by MoAb M35 in western blots. A 20 kDa HK endoprotease- and trypsin-resistant ligand-binding site of keratinocyte mAChR was detected in the SDS-PAGE gels using irreversible muscarinic antagonist [^{3}H]propylbenzilylcholine mustard.

Thus, the use of MoAb M35 allowed us to demonstrate localization of mAChR in human epidermis and HK cultures and further characterize the biology and structure of the receptors.

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DYNAMIC REGULATION OF ß-ADRENORECEPTORS OF KERATINOCYTES BY GLUCOCORTICOIDS.

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Normal human keratinocytes (NHK) reveal high levels of ß-adrenoreceptors (ß-AR), predominantly of the ß2-subtype. Moreover, ß-AR couple via G-proteins to the intracellular adenylate cyclase-cAMP-system, as demonstrated by pronounced cAMP accumulation after exposure to beta-agonists. Finally,this signal transduction increases the intracellular calcium concentration. In psoriasis and atopic eczema dysfunction of ß-AR regulation have been discribed. Glucocorticoids (GC) are potent anti-inflammatory drugs, but their mechanism of action is still not fully understood. Autoradiographic studies showed an significant up-regulation of ß-AR in normal as well as in atopic skin after topical application of GC.

The aim of this study has been to determine the influence of GC on the ß-AR-density of NHK in vitro. Keratinocytes from neonatal foreskin were established in MCDB-derived serum-free medium and grown to confluency. Binding experiments were performed with saturating levels of 3H-CGP-12177, a beta-adrenergic antagonist.

Untreated NHK express about 7,000 ß-AR/cell. Incubation of NHK with dexamethasone (10µM) leads to a profound increase of ß-AR up to 130% (6-12hs), slowly decreasing to 50% (24hs) and 30% (48hs). Using accelerating concentrations of dexamethasone (1M-1µM), distinct differences in the degree of up-regulation, ranging from 80% (1µM) to 10% (100µM) and even down-regulation of 35% (1M) could be observed. In contrast, the beta-agonist isoprenalin leads to dramatic reduction of ß-AR-density of 80%.

Our data demonstrate the GC-induced regulation of ß-AR of NHK as a dynamic, time and concentration dependent process. Further studies are necessary to elucidade wether this up-regulation may be of functional relevance for the anti-inflammatory actions of GC.

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L-36 MAMMALIAN LACTOSE-BINDING LECTIN IS AN EPITHELIAL JUNCTION PROTEIN

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We have described a 37 kDa pig epithelial junction protein, probably an adherens junction protein, which is unique because of its limitation to suprabasal cells and its restriction, in the case of stratified epithelium, primarily to oral epithelium. cDNA cloning from a pig tongue cDNA library yielded a 1.1 kb full length clone coding for a 324 amino acid protein which on homology search was found to be 71% identical with a rat intestinal lectin of unknown function known as L-36. It was not clear whether the pig protein was functionally similar homolog or a closely related protein with a different function. Attempts to demonstrate the protein in human and rat epithelium and in cultured pig oral keratinocytes with polyclonal antibody to the pig protein were unsuccessful. Since the rat protein is present in intestine, we stained cultured human colon carcinoma cells with antibody to the pig protein. Staining revealed typical junctional staining very similar in location to E-cadherin (uvomorulin), an adherens junction protein. Furthermore, reverse transcriptase-PCR using human tongue mRNA or human colon carcinoma cells yielded cDNAs of 290 and 590 bp respectively with 80 %identity in deduced amino acid sequence to the rat and pig proteins. Since both pig and human proteins have the typical appearance of junction proteins by immunofluorescence; we conclude that the L-36 protein is a junction protein and that the mammalian junction protein is a lactose-binding lectin.

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ROLE OF GLUCOSYLATION OF CERAMIDE IN EPIDERMAL BARRIER FORMATION.

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We have investigated the physiological significance of glucosylation of ceramide in the synthetic pathway of acylceramide, an important ceramide species for epidermal barrier, by examining the metabolic pathway of [^{14}C]-labelled serine in mouse epidermal tissue culture system and by determining the substrate specificity of glucosyltransferase (UDP-glucose:ceramide glucosyltransferase) *in vitro*.

Cultured new born mouse (Balb/c) epidermal tissue synthesized several types of [^{14}C]-labelled ceramides including ceramide type 1 or acylceramide as analyzed by TLC followed by autoradiography. When Br-CBE (bromocondritol-B-epoxide), an inhibitor of glucocerebrosidase, was added in the culture medium, synthesis of acylceramide was inhibited completely while other types of ceramides remained unchanged. Furthermore, addition of PDMP (D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol), an inhibitor of glucosyltransferase, also specifically abolished the synthesis of acylceramide, indicating that glucosylation of ceramides was an essential requirement for acylceramide synthesis.

We further determined if the physiological substrate of glucosyltransferase was omega-hydroxylceramide (C30) or acylceramide (linoleoyl C30 ceramide). Neither of them was good substrate for glucosyltransferase as measured *in vitro*, suggesting that glucosylation occurs before the formation of omega-hydoroxylceramide although the mechanism of omega-hydroxylceramide synthesis remained to be clarified.

These findings demonstrate for the first time that acylceramide found in stratum corneum is synthesized through definite successive processes consisting of glucosylation of ceramide, acylation of glucosylceramide and de-glucosylation of acylglucosylceramide.

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STRUCTURE DETERMINATION OF ACYLGLUCOSYLCERAMIDES OF HUMAN CULTURED KERATINOCYTES.

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Acylglucosylceramides(AGC) are important glycosphingolipids of the epidermal barrier of transepidermal water loss. In human epidermis, AGC contain only linoleic acid for ester linked fatty acid(epidermosides). AGC are rich in granular cells and disappear in horny cells, suggesting that their expression is closely related to epidermal cell differentiation. Therefore, we cultured human keratinocytes(CHK) which did not differentiate into granular or horny cells and analyzed their glycosphingolipids.

AGC were isolated by DEAE and silica gel column chromatographies, further by HPLC on a silica gel column, and analyzed by means of ^{1}H-NMR spectroscopy, fast atom bombardment(FAB) mass spectrometry and GLC-mass spectrometry. In CHK, the AGC content was lower than that of human epidermis. The ^{1}H-NMR spectrum indicated that the linoleic acid content of AGC from CHK was very low. Major molecular weights determined by negative-ion FAB mass spectrometry were 1173 and 1201. AGC were consisted of amide linked ω-hydroxy fatty acids (C30:0, C30:1, C32:1 and C34:1), fatty acids linked to the ω-hydroxy fatty acids through ester linkages (C14:1, C16:1, C18:1 and C18:2), a long chain base(d18-sphingenine), and β-glucose. AGC containing phytosphingosine was not detected. AGC biosynthesis may not be fully activated in CHK under the culture conditions we used and full activation may be achieved in more differentiated keratinocytes, such as spinous cells at a late stage or granular cells.

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EXPRESSION AND FUNCTION OF NERVE GROWTH FACTOR AND NERVE GROWTH FACTOR RECEPTORS ON CULTURED NORMAL HUMAN KERATINOCYTES. Carlo Pincelli, Rossella Manfredini, Cinzia Sevignani, Luisa Benassi, Fabrizio Fantini, Luigi Aloe, Sergio Ferrari and Alberto Giannetti. Institute of Dermatology and Hematology, University of Modena and Institute of Neurobiology, C.N.R., Rome, Italy.

Nerve growth factor (NGF), in addition to its neurotrophic properties, possesses a number of biological effects on several cell types. NGF receptor (NGF-R) has been recently detected in normal human skin by immunohistochemistry. In the present study we evaluated function and expression of NGF and NGF-R on normal human keratinocytes (NHK) cultivated on 3T3 cells. Using the reverse-transcription polymerase chain reaction (RT-PCR), both the low- and the high-affinity (trk) NGF-R mRNA were detected on NHK. Addition of either NGFbeta or NGF 2.5S (10, 100, 500 ng/ml) to the medium significantly stimulated the proliferation of NHK in a dose dependent manner (NGFbeta = 25, 55, 75 % cell number increase respectively, $p< 0.01$; NGF 2.5S = 10, 35, 60 % cell number increase respectively, $p< 0.05$). The NGF-induced keratinocyte proliferation was significantly blocked by anti-NGF neutralizing mAb. Furthermore, NGF mRNA was detected in NHK by RT-PCR and NGF was shown to be released by NHK, as measured by ELISA. In particular, NGF levels (pg/ml) were secreted in increasing amounts during proliferation (day 1=96±33; day 5=140±31), whereas they began to decrease after confluence (day 7=64.4±33.6; day 8=45.1±7.6; day 13=21.4±4.4). These results indicate that NGF might have an important function in human skin and suggest the hypothesis that NGF plays an autocrine role in the proliferation of NHK.

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CHARACTERIZATION OF THE S-100-LIKE CALCIUM BINDING DOMAIN OF HUMAN PROFILAGGRIN. R.B. Presland, J. R. Kimball and B.A. Dale. Dept. of Oral Biology, University of Washington, Seattle, WA, U.S.A.

Filaggrin (FG) is the intermediate-filament associated protein which functions to aggregate keratin IFs in the stratum corneum of epidermis. It is synthesized as a large precursor protein, profilaggrin (proFG), that consists of multiple FG repeats and is localized in keratohyalin (KH). We recently reported the genomic organization of the human proFG gene and identified an S-100-like EF hand domain in the first 81 amino acids of the predicted protein sequence (*JBC 267*, 23772-81, 1992). To study its possible role in the processing of proFG to FG, a peptide antibody (EF-Ab) directed against part of the first EF hand (aa 16-30) was prepared and used for immunohistochemical and Western analysis of human and mouse epidermis. The EF-Ab reacts strongly with cells in the upper granular and transition layers where proFG is being processed, but only weakly with KH. There was no detectable staining of other epidermal cell layers or the cornified cell envelope. The EF-Ab reacts weakly with proFG, strongly with polypeptides of 50-55 and 16.5 kD, and less intensely with bands of 90 kD and ~150 kD. They may represent processing intermediates of proFG or non-specific proteolytic products. To study calcium binding, the 293 aa N-terminal domain was expressed in *E. coli* and calcium binding was analyzed by the $^{45}Ca^{2+}$ overlay technique. Results obtained with whole cell bacterial extracts suggests that the 36 kD recombinant protein binds calcium, although the binding is weak compared to yeast calmodulin which contains three functional EF hands. We hypothesize that the N-terminal EF hand domain of proFG is critical for proFG expression or processing to FG.

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EXPRESSION OF CORNIFIN IN SQUAMOUS DIFFERENTIATED EPITHELIAL TISSUES. Wataru Fujimoto, Keith W. Marvin*, Anton M. Jetten*, Jiro Arata, Department of Dermatology, Okayama University Medical School, Okayama, Japan. *Cell Biology Section, Laboratory of Pulmonary Pathobiology, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC, U.S.A.

Cornifin is a putative cross-linked envelope precursor in keratinocytes. In order to examine the expression and regulation of cornifin in vivo, we investigated several squamous differentiated tissues by in situ hybridization and immunohistochemical analysis. Cornifin mRNA and protein, which are absent in the normal mucociliary epithelium, are induced in the suprabasal layers of the squamous metaplastic tracheal epithelium of vitamin-A deficient hamsters. Similar to the induction of squamous metaplasia in vivo, culture of rabbit tracheal cells in the absence of retinoids results in squamous differentiation and expression of cornifin. This induction of cornifin expression is suppressed by retinoic acid and several of its analogs. Cornifin mRNA and protein are also detected in the suprabasal layers of the squamous epithelium of rabbit esophagus and tongue. The distribution of cornifin in human epidermis was compared with that of two other cross-linked envelope precursor proteins, involucrin and loricrin. Cornifin and involucrin are induced in the spinous layer and appear at an earlier stage during epidermal differentiation than loricrin. The expression of cornifin is greatly increased in psoriatic skin. Cornifin mRNA is barely detectable in normal epidermis whereas it is present at relatively high levels in the suprabasal layers of psoriatic epidermis. Topical treatment with RA results in thickening of the skin and increases the level of cornifin mRNA and protein in the upper spinous layers of mouse skin. In conclusion, cornifin expression correlates generally with squamous differentiation in a variety of tissues and is abnormally regulated in psoriatic skin and in skin treated topically with retinoic acid.

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EPIDERMAL EXPRESSION OF PROFILAGGRIN PHOSPHATASE, A PROTEIN PHOSPHATASE TYPE 2A. B.A. Dale, E. Kam, A. Glass, R. Presland, Depts. of Oral Biology, Periodontics, and Medicine/Dermatology, Univ. of Washington, Seattle, WA, USA.

Epidermal granular cells undergo an abrupt transition to fully differentiated cornified cells lacking organelles and filled with densely packed keratin proteins. During this transition the keratohyalin protein, profilaggrin, is dephosphorylated and proteolytically processed to filaggrin which aggregates keratin filaments. In order to better understand this process, a profilaggrin phosphatase was characterized from rat epidermis. This enzyme is a phosphatase type 2A (PP2A) not dependent on divalent cations and inhibited by okadaic acid. PP2A is a highly conserved serine/threonine protein phosphatase made up of one catalytic subunit and two regulatory subunits. We studied the expression of both the protein and mRNA of the PP2A catalytic subunit. Using an antibody (a gift of M. Mumby), we identified a band of the expected size (36 kDa) by Western analysis of human epidermal extracts. Immunostaining gave a strong reaction in the granular layer where profilaggrin is first expressed and then processed. Probes specific for the alpha and beta isoforms of the PP2A catalytic subunit were prepared and used for Northern analysis and for *in situ* hybridization. Both isoforms are expressed in epidermis and cultured keratinocytes and are strongly expressed in the granular cell layer. The PP2A catalytic subunit beta was cloned from an epidermal cDNA library and cloned into the pET-15b vector for expression in *E. coli*. The fusion protein is being purified and tested for phosphatase activity with phosphofilaggrin. Optimization of expression will enable detailed studies of the regulation of this enzyme and its role in profilaggrin processing and keratinization.

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ESSENTIAL ROLE OF EICOSANOID PRODUCTION AS AN ONCOGENE INDUCER IN INSULIN-LIKE GROWTH FACTOR-I STIMULATED MITOGENESIS OF HUMAN KERATINOCYTES

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Little is known about an early event in mitogenic cascade induced by growth factors in human keratinocytes. We investigated a possible involvement of eicosanoids as an oncogene inducer in growh factor-stimulated mitogenesis. Insulin-like growth factor-I (IGF-I, 10-100 ng/ml) induced a significant and dose-dependent increase of DNA synthesis (thymidine incorporation) in cultured human keratinocytes, accompanied by marked and simultaneous secretion of prostagrandin E2, but not for leukotriene C4. This growth stimulatory effect was curtailed by the prior and concomitant addition of a cyclooxygenase inhibitor, indomethacin (IM), but not by a lipoxygenase inhibitor, AA861. Furthermore, though western blots for IGF-I stimulated human keratinocytes revealed that production of c-myc encoded protein (MW; 67kDa) markedly increased with a maximum at 1-2 h after incubation with IGF-I, but the pretreatment by IM significantly suppressed the production. On the other hand, IM failed to inhibit tyrosine autophosphorylation of IGF-I receptor by IGF-I, indicating no direct action of IM toward IGF-I receptor. These findings suggest that prostagrandins are involved as an oncogene inducer in the IGF-I stimulated mitogenesis of human keratinocytes.

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TISSUE-SPECIFIC AND DIFFERENTIATION-APPROPRIATE EXPRESSION OF THE HUMAN INVOLUCRIN GENE IN TRANSGENIC MICE - A ROLE FOR INVOLUCRIN IN THE HAIR FOLLICLE AND EPIDERMIS Richard L. Eckert, Shubha Murthy, James F. Crish, Tarif M. Zaim, Depts of Physiology and Biophysics, Dermatology, Reproductive Biology and Biochemistry, Case Western Reserve University School of Medicine, Cleveland, Ohio

Involucrin is an important precursor of the keratinocyte cornified envelope. To study the regulation of the involucrin gene and the function of involucrin, we have expressed human involucrin (hINV) in mice. The transgene produces a 68 kDa, anti-involucrin immunoreactive protein that displays correct tissue-specific and differentiation-appropriate expression (suprabasal staining of esophagus, cervix and epidermis). An important finding is the presence of hINV in hair follicle structures including the medulla, cuticle and inner root sheath. The distribution of hINV is comparable in human and transgenic mice. The epidermis of transgenic animals displays changes in morphology including epidermal scaling and an abnormal hair coat that appears to be caused by hINV over-expression. Our results indicate that the transgene contains all of the sequence required for appropriate regulation and suggest a new involucrin function as a cross-linked constituent of IRS cell membrane. These animals provide a new model for the study of hINV gene regulation and function.

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HIGHLY DYNAMIC PROPERTIES OF KERATIN INTERMEDIATE FILAMENTS IN VIVO AND IN VITRO. P.M. Steinert, Skin Biology Branch, NIAMS, NIH, Bethesda, MD. and R.D. Goldman, CMS Biology, Northwestern University Medical School, Chicago, IL.

By use of crosslinking experiments, we have recently determined the axial dimensions and alignments of coiled-coil molecules in keratin intermediate filaments (KIF). Interestingly, the molecules overlap in certain key sequence regions, including the H1 end domain region, the beginning of 1A and the end of the 2B rod domain segments. Here we demonstrate that synthetic peptides corresponding to these sequences can markedly affect the structures of preformed KIF in vitro, and the cytoskeletal organization of KIF when micro-injected into living cells. Peptides of 15-45 residues corresponding to these sequences of the human K1 chain were synthesized and purified by HPLC. When mixed with preformed KIF in vitro at a 1:1 molar ratio, each peptide resulted in a rapid disassembly of the KIF, down to single coiled-coil molecules, and was reversible on removal of the peptide. Interestingly, mixtures of any combination of two peptides protected each other from dis-assembly. Micro-injection of the 1A and 2B peptides also caused catastrophic but reversible disassembly of the endogenous IF networks of living epithelial or fibroblastic cells, but the H1 peptide specifically destroyed only KIF networks. The peptides do not alter the organization or state of assembly of microtubules or F-actin stress fibers in cultured cells. These data indicate that the IF are far more dynamic in solution and in living cells than previously thought. The peptides apparently compete with and displace the full-length protein chains in the IF, promoting collapse of IF structure. Control peptides involving either other sequences of the K1 chain, or sequences unrelated to keratins, did not affect IF integrity in vitro or in vivo. We have also found that synthetic peptides containing the disease-causing amino acid substitutions reported in epidermolysis bulosa simplex and epidermolytic hyperkeratosis have a markedly reduced affect on preformed KIF in vitro, presumably because the substitutions seriously alter structure and function. Peptides thus offer (i) a convenient way to explore the organization and function of IF in living cells or tissues and their interactions with other macromolecules; and (ii) afford a simple functional test for disease-causing mutations found in pathology.

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THE LARGE TYPE II 79kDa KERATIN DISPLAYING BODY SITE SPECIFIC EXPRESSION IN MOUSE EPIDERMIS IS THE ORTHOLOG OF HUMAN KERATIN K2e. Frank Herzog, Hermelita Winter and Jürgen Schweizer, Research Program II, German Cancer Research Center, Heidelberg, FRG

The basic keratin pattern of mammalian epidermis consists of the basal keratin pair K5/K14 and the differentiation-specific keratin pair K1/K10. Distinct skin sites of the adult mouse, i.e. ear, sole of the foot and interscale regions of tail skin express, an additional, type II 70kDa keratin without a defined new type I partner in suprabasal epidermal cells. Up to now, the question whether this large keratin is specific for the mouse (or related small rodents) or whether orthologous keratins exist in other species has not yet been answered. We have therefore determined the full length amino acid sequence of the 70kDa keratin which comprises 707 amino acid residues and has a calculated molecular weight of 70976.70 Da. Structurally, the 70 kDa keratin is remarkable in that more than half of both its V1 and V2 subdomains of the head and tail portions consists of different glycine-rich peptide motifs that are configured consecutively at least two times and as much as seven times in tandem. By means of sequence comparisons and phylogenetic investigations we show that the 70kDa keratin represents the murine ortholog of the human 65kDa keratin K2e which also exhibits a restricted expression pattern and whose nature as a genuine keratin has recently been demonstrated (Collin et al. Exp. Cell res. 202:132-141, 1992). Whereas size differences between orthologous keratins of the basic epidermal keratin pattern are generally in the range of 1-2 kDa, the orthologous keratins MK2e and HK2e are remarkable by their unusually large size difference of 5kDa. We show that this property is due to a different duplication rate of the glycine-rich peptide motifs in the respective V subdomains of these highly specialized keratins. The identification of MK2e and HK2e as orthologs stengthens the view of uniform mammalian keratin repertoire and lowers the probability of the existence of species-specific keratins in mammals.

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REGULATION OF KERATIN GENE EXPRESSION BY HORMONES, VITAMINS, GROWTH FACTORS AND CYTOKINES. Miroslav Blumenberg, Marjana Tomic-Canic, Chun-Kui Jiang, Shawna Flanagan, Madeline Krauss and Irwin Freedberg, Ronald O. Perelman Department of Dermatology, NYU Medical Center, New York, USA

The extracellular milieu regulates the expression of keratin genes via two types of receptors, nuclear and cell surface. Retinoids, important therapeutic agents in epidermis, function via nuclear receptors, and so do vitamin D3, thyroid and steroid hormones. We have made use of mutant receptors as well as site-directed mutagenesis of the recognition elements in keratin genes. These results show that the regulation of keratin genes by nuclear receptors proceeds by a direct mechanism, by binding of the nuclear receptors to their cognate DNA recognition elements via the DNA-binding domains and interaction with the transcriptional machinery via the ligand-binding domains. Growth factors and cytokines, on the other hand, work via cell surface receptors. For example, TGFβ profoundly effects keratinocytes by blocking their proliferation in vitro. TGFβ-treated keratinocytes remain viable and show no signs of differentiation. To investigate the molecular mechanisms by which TGFβ affects keratinocytes, we have transfected keratinocytes with DNA constructs in which promoters of keratin genes drive expression of the CAT reporter gene. The transfected cells were grown in the presence or absence of TGFβ. We found that TGFβ has no effect on the promoters of genes K#3, K#6, K#8, K#10, K#16, K#17, K#18 and K#19. However the basal cell-specific keratin promoters K#5 and K#14 are induced. By deletion analysis, we have mapped the TGFβ-responsive elements to short segments in each of the regulated genes. Our data suggest that TGFβ promotes a basal cell phenotype and that its antiproliferative effect may be at the expense of the rapidly dividing, activated keratinocytes.

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MOLECULAR MECHANISMS OF KERATINOCYTE- AND DIFFERENTIATION-SPECIFIC EXPRESSION OF THE 230-KDa BULLOUS PEMPHIGOID ANTIGEN GENE (BPAG1). Katsuto Tamai, Kehua Li, Stephanie Silos, and Jouni Uitto, Department of Dermatology, Jefferson Medical College, Philadelphia, PA

We have recently cloned the entire human BPAG1 gene, including the functional promoter. Transfections of the promoter-CAT constructs suggested the existence of *cis*-elements responsible for keratinocyte-specific expression within 1.9 kb of 5' flanking DNA upstream from the transcription initiation site. First, a putative AP-2 binding sequence (KRE2), which was found in the position -[1887 to 1898], was shown to upregulate the expression of the BPAG1 promoter in cultured human keratinocytes. Keratinocyte nuclear extracts contained an abundant protein, designated as KTP-1, which complexed with the KRE2, when studied by the DNA mobility shift assay. KTP-1 was clearly distinct from AP-2 by molecular size, the sequence specificity and subunit composition requirement for DNA binding. Secondly, a strong upregulatory region for BPAG1 expression in cultured keratinocytes, but not in fibroblasts, was identified in the region -[277 to 397] , and deletion of this sequence abolished the expression in keratinocytes. Finally, a Ca^{2+}-responsive element was located to the region -[2,536 to 1,806]. Increase of Ca^{2+} concentration from 0.15 to 2.0 mM resulted in >80% suppression in the activity of the promoter containing this DNA sequence. These data indicate that the BPAG1 promoter has at least two distinct *cis*-elements necessary for keratinocyte-specific expression, and a separate response element may be responsible for differentiation-specific down-regulation of the BPAG1 gene expression in keratinocytes.

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cDNAS ENCODING TWO CHAINS OF THE ANCHORING FILAMENT PROTEIN KALININ SHOW SIMILARITY TO LAMININ B1, AND B2. D.R. Gerecke, D.W. Wagman, M-F Champliaud, and R.E. Burgeson, Dept. Dermatology, Mass. Gen. Hospital, Harvard Medical School, Boston, MA

In order to maintain epidermal attachment to the dermis, skin and other external tissues have evolved a secondary attachment mechanism collectively termed the *anchoring complex*. Ultrastructurally this complex consists of a number of distinct structures including *hemidesmosomes, anchoring filaments,* and *anchoring fibrils.* A disruption of any of the components of the anchoring complex could result in clinical minifestations. In fact, some of the subtypes of the family of diseases, *epidermolysis bullosa*, have been correlated to mutations in the complex. Kalinin is one of the proteins believed to be localized in the anchoring filaments. Kalinin is composed of three nonidentical chains 165, 155, and 140 kD in size (Rouselle *et al., J.C.B.* 114:567-576, 1991). Using anti-kalinin polyclonal serum, we have screened a human squamous carcinoma cell lgt11 library and isolated a number of cDNAs corresponding to two chains of kalinin. Deduced amino acid sequences from the cDNAs encoding the 140 kD chain indicate it is related to the previously described human laminin B1 chain, but is truncated in comparison to laminin B1. Deduced amino acid sequences from the cDNAs encoding the 155 kD chain indicate it is related to the previously described human laminin B2 chain. This suggests that kalinin is a laminin isotype. Since molecules that have very similar amino acid sequences often have similar functions, we predict that kalinin and laminin will have similar functions, and, in fact, like laminin, kalinin is involved in attachment of epithelial cells to the basement membrane. However, the two proteins probably do not have identical functions, since some of the functional domains that have been attributed to the B1 and B2 chains in laminin are absent in their kalinin counterparts.

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PHARMACOLOGICAL REGULATION OF SWEAT DUCTAL FUNCTION AS STUDIED BY WHOLE CELL VOLTAGE/CURRENT CLAMP METHODS. K. Sato, M. Ohtsuyama, and F. Sato. Marshall Dermatology Research Laboratories, University of Iowa College of Medicine, Iowa City, IA, USA.

Absorption of NaCl for conservation of the vital electrolytes is the principle function of the eccrine duct, yet the pharmacological basis of ductal function has been totally unknown. It has been puzzling how a sweat duct that lacks direct innervation can be functionally regulated and how the regulation of the two cell layers of the ductal epithelium is coordinated. Eccrine sweat ducts from rhesus palm eccrine sweat glands were isolated and subjected to collagenase digestion. The dissociated luminal ductal cells could be visually distinguished from the basolateral ductal cells because of distinctive differences of morphology and cell size. The membrane potential (PD) as studied by whole cell current clamp method was comparable between luminal and basolateral ductal cells, ranging from -80 to -150 mV. Isoproterenol (ISO, at 10 μM) + 1 mM ATP-γS depolarized the PD by 40 to 85 mV in both luminal and basolateral cells. ISO+ATP-induced depolarization was associated with an increase in membrane conductance and Cl current as studied in physiological conditions and TrisCl (bath)/CsCl (intracellular and pipet) system, suggesting that Cl channels were predominantly activated. Ionomycin (Ca ionophore, at 10μM) mimicked the effects of ISO and ATP. Methacholine (MCh) caused a transient depolarization in a small number of basolateral cells. Interleukin (IL)-8 at 10 ng/ml drastically depolarized PD but only in luminal ductal cells, i.e., IL-8 had no effect on basolateral cells or secretory cells. IL-1 and IL-6 at 5-10 ng/ml also had no effect on luminal or basolateral cells. IL-8-induced depolarization of luminal ductal cells was associated with inward current which was most likely due to Cl current because IL-8 also stimulated membrane current in the absence of Na inside and outside the cell. The observation raises a possibility that the ductal NaCl absorption is regulated by IL-8 generated de novo by the cytokine cascade by stimulating Cl channels. The identity of these IL-8 stimulated Cl channels is unknown.

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CHARACTERIZATION OF MUTATIONS IN THE TYPE VII COLLAGEN GENE IN PATIENTS WITH THE DYSTROPHIC FORMS OF EPIDERMOLYSIS BULLOSA. Angela M. Christiano, *Eugene A. Bauer and Jouni Uitto. Departments of Dermatology, Jefferson Medical College, Philadelphia, PA; and *Stanford University, Stanford, CA.

Dystrophic epidermolysis bullosa (EB) is a mechanobullous disorder of the skin in which the anchoring fibrils of the basement membrane zone, composed of type VII collagen, are absent or severely abnormal. We have previously demonstrated strong genetic linkage between the type VII collagen gene (COL7A1) at the chromosomal locus 3p21 and both the dominant and recessive forms of dystrophic EB. We have also recently completed cloning and characterization of the entire gene and full-length cDNA for type VII collagen. To facilitate the detection of the underlying mutations in patients with dystrophic EB in the gene, we designed overlapping PCR amplimers which span the entire genomic sequence. The first homozygous missense mutation in exon 113 of COL7A1 was identified in two siblings with a mild form of recessive dystrophic EB using SSCP analysis (Christiano et al., Nature Genet. 4:62-66). Recently, we identified two mutations in another patient with severe mutilating (Hallopeau-Siemens) receissive dystrophic EB using MDE analysis. This patient is a compound heterozygote, with a maternally inherited insertion of 1 bp in exon 19 on one allele, and a paternally inherited deletion of 1 bp in exon 31 on the other allele. Delineation of the underlying mutations in the dystrophic forms of EB will facilitate the design of gene therapy modalities to reverse the effects of this devastating disease of the skin.

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ULTRAVIOLET B EXPOSURE INDUCES INTERLEUKIN-10 EXPRESSION IN HUMAN KERATINOCYTES. Claes D. Enk and Stephen I. Katz, NIH, Beth, MD.

Interleukin-10 (IL-10) inhibits proliferation and cytokine production by T helper-1 lymphocytes and suppresses delayed type hypersensitivity reactions while facilitating allergic/humoral responses. We have previously shown that murine keratinocytes produce IL-10 mRNA and protein after exposure to contact allergens. To determine the presence of IL-10 mRNA in human keratinocytes and to study the regulation of IL-10 gene transcription by UVB, we isolated mRNA from cultured human keratinocytes at different times after exposure to varying intensities of radiation. Reverse transcriptase-PCR with primers for IL-10 (or beta actin, as a control) was used to amplify mRNA, then PCR products encoding IL-10 were identified on polyacylamide gels after liquid hybridization with end-labeled oligonucleotide internal probes. Whereas non-irradiated cultured keratinocytes expressed no detectable IL-10 message, UVB radiation (100 J/m^2 and 200 J/m^2) induced IL-10 signals 6 and 24 h post-irradiation. To determine whether UVB induces IL-10 transcription in vivo, we similarly analyzed mRNA from human epidermal cells by removing tops of suction blisters that were induced 18 h after exposure to 4 MED UVB radiation. Although IL-10 mRNA was expressed in non-UVB exposed epidermis in some individuals, it was markedly enhanced after UVB radiation. These data demonstrate that IL-10 gene expression in cultured human keratinocytes is inducible by UVB in vitro and that keratinocyte IL-10 gene expression is also enhanced by UVB in vivo. We propose that human keratinocyte-derived IL-10 may regulate inflammatory skin reactions and be responsible for some of the immunosuppressive properties of UVB.

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INTERLEUKIN-6 ENHANCES CORNIFIED ENVELOPE FORMATION IN THE STRATIFIED KERATINOCYTES CULTURED IN A NORMAL CA^{2+} CONCENTRATION. Y. Mitsuhashi, Y. Takagi, H. Ishikawa and I. Hashimoto. Department of Dermatology, Hirosaki University School of Medicine, Hirosaki, Japan

IL-6 is a multifunctional cytokine which is believed to act as a proliferator for keratinocytes. However, this consensus depends on the in vitro observation in which keratinocytes were cultured with serum free in a low Ca^{2+} concentration (less than 0.1mM). It is not clear whether IL-6 proliferates keratinocytes in a normal Ca^{2+} concentration with adding of serum, which is a condition much closer to in vivo. To elucidate how IL-6 effects proliferation and differentiation of keratinocytes in a normal Ca^{2+} condition, we made a cell culture experiment. Normal human keratinocytes were cultured on a permeable collagen membrane with Eagle's minimum essential medium with supplement of antibiotics and 10% of fetal calf serum. Concentration of Ca^{2+} in the medium was 1.4mM. When they reached confluent, sequential doses (0, 0.5, 4.0, 20 and 100ng/ml) of human rIL-6 were added in the medium. After 12 days of culture with IL-6, the stratified epithelium was harvested and the amounts of total protein, DNA and cornified envelope were quantified. The results show that the amounts of protein and DNA were not different among any doses of IL-6. However, formation of cornified envelope was increased depending on the dose of IL-6 from 0 to 20ng/ml. Cornified envelope formation in the condition of 100ng/ml of IL-6 was suppressed to the same level as a control culture. These results suggest that IL-6 acts as a differentiator on stratified keratinocytes in a normal Ca^{2+} condition.

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OVEREXPRESSION OF INTERLEUKIN-8 RECEPTOR IN PSORIATIC EPIDERMIS. EFFECT OF NOVEL ANTIPSORIATIC AGENTS. Thomas Ruzicka, Barbara Schulz, Axel Beetz, Lajos Kemény, Günter Michel, Depts of Dermatology, Univ. of Munich, Germany, Univ. of Szeged, Hungary.

Interleukin-8 (IL-8) is supposed to play a role in the pathogenesis of psoriasis and is known to exert its effects via specific receptors. Recently we could for the first time demonstrate IL-8 receptors (IL-8R) on human keratinocytes (KC). We therefore studied the expression of epidermal IL-8 and IL-8R in psoriatic skin and their regulation by antipsoriatic substances.

Keratoma biopsies were obtained from lesional and non lesional skin of eight chronic plaque type psoriatics and from normal skin. Gene expression was monitored using a semiquantitative reverse-transcriptase PCR technique.

There were no IL-8 specific signals in healthy and uninvolved epidermis, but markedly elevated transcript levels in psoriatic skin. The level of IL-8R mRNA was found to be about 10-fold increased in involved psoriatic skin whereas only weak signals were obtained in uninvolved and normal skin. Leukocytes were excluded as major source of IL-8R increase by determination of elastase mRNA and histology. In experiments designed to analyse the pharmacological regulation of IL-8 binding to KC, the novel antipsoriatic agents calcipotriol and FK 506 proved to be potent inhibitors of IL-8R expression.

In conclusion, psoriatic skin shows massive overexpression of the IL-8R, which may serve as a target for antipsoriatic agents.

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IL-8 RECEPTOR AND P53, BUT NOT PROTOONCOGENES C-RAF AND C-RAS, ARE POTENTIAL TARGETS OF FK 506 IN PSORIASIS. Günter Michel, Christine Ried, Axel Beetz, Lajos Kemény, Thomas Ruzicka; Depts. of Dermatology, University of Munich, Germany, University of Szeged, Hungary

The microbial macrolide FK 506 exhibits very potent antipsoriatic properties besides its immunosuppressive effects. Since FK 506 is known to act at the level of gene expression, we asked if the drug modulates genes that are believed to be important for the pathogenesis of psoriasis. Recently we could demonstrate aberrant overexpression of IL-8 as well as its receptor in psoriatic skin by mRNA-PCR. Therefore we now analysed the expression of these genes in epidermal cells under the influence of FK 506. Since psoriasis is characterized by uncontrolled epidermal growth we also studied the effects of the drug on the expression of the protooncogenes c-ras and c-raf, which code for mitogenic signal transduction components, and of the growth inhibitory gene p53.

Human foreskin keratinocytes were incubated in the presence of FK 506. Gene expression was monitored by semiquantitative mRNA-PCR. Binding characteristics were studied by flow cytometry using phycoerythrin-labelled IL-8.

After 3h the IL-8R mRNA levels were dose-dependently decreased in FK 506 treated cells to 30% of untreated controls. Binding of IL-8 to the cells was also dose-dependently inbibited after 24h. Less pronounced inhibition was seen with IL-8 mRNA, whereas protooncogene expression was not significantly altered. Interestingly a massive induction of p53 by a factor of 2.5 was elicited by FK 506 treatment.

These results suggest that IL-8R and the growth inhibitor p53 represent potential targets for the antipsoriatic action of FK 506, wheras protooncogenes involved in mitogenic signal transduction are unaffected. The inverse regulation of the receptor for the mitogenic cytokine IL-8 and the growth inhibitor p53 provides evidence for the specificity of the drug effects and rules out nonspecific toxic effects on KC.

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CYTOKINE GENE POLYMORPHISMS IN INFLAMMATORY DERMATOSES. M.J. Cork*, J.K. Tarlow, A.G. Wilson, A.I.F. Blakemore, G. Messer+, P. Kind+, C. Gordon@, P. Emery@, S.S. Bleehen*, and G.W. Duff. Sections of *Dermatology and Molecular Medicine, Department of Medicine and Pharmacology, Royal Hallamshire Hospital, Sheffield, S10 2JF, UK. @Dept. of Rheumatology, University of Birmingham, UK. +Dermatologische Klinik und Poliklinik, der Ludwig -Maximilians-Universitat, Munchen, Germany.

Inflammatory dermatoses such as SLE, dermatitis herpetiformis (DH) and psoriasis are probably polygenic. We would therefore expect to see small changes in the allelic frequencies of several genes. The numerous abnormalities of cytokine production, receptor expression and inhibitor levels demonstrated in these diseases have not previously been related to their genetic component. We postulated that polymorphisms in the regulatory regions of cytokine and related genes may provide this link.

We have characterised a 5-allele polymorphism due to a variable number of an 86bp tandem repeat in intron 2 of the IL-1 receptor antagonist (IL-1ra) gene. Mendelian segregation of the alleles has been demonstrated in four extensive pedigrees. We have determined allelic frequencies for the IL-1ra polymorphism in a control population and compared this with that in SLE, DH and psoriasis. Allele 2 had a frequency of 24.1 % in the control population. This was increased to 60% in patients with SLE and discoid rash and 40% in those with SLE and photosensitivity. There was a direct relationship with disease severity from 25% (ARA criteria =4) to 48% (ARA=8). Allele 2 was also increased in type 1 psoriasis and DH. We have also demonstrated a biallelic polymorphism in the TNF-alpha promoter region. Frequency analysis of this polymorphism revealed a very strong association between the uncommon TNF allele and HLA A1, B8 and DR3 alleles. The frequency of the rare allele was increased to >50% in patients with DH. We are currently performing functional studies to determine if the rare alleles of the IL-1ra and TNF-alpha polymorphism are responsible for low and high production respectively.

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KERATINOCYTE-DERIVED IL-7 UPREGULATES TCR/CD3 EXPRESSION BY DENDRITIC EPIDERMAL T CELLS. M Ono, K Ariizumi, PR Bergstresser, A Takashima. UT Southwestern Medical Center, Dallas, TX, USA and Kawasaki Medical School, Kurashiki, Japan

Dendritic epidermal T cells (DETC) in adult mouse skin express the phenotype Thy-1$^+$, TCR-Vγ3/Vδ1$^+$, CD3$^+$, CD4$^-$ and CD8$^-$. Thy-1$^+$ cells in newborn mice, however, rarely express Vγ3/Vδ1 TCR or CD3, indicating that phenotypic maturation and/or rapid expansion of Vγ3/Vδ1$^+$ cells must occur within the skin after birth. We observed recently that keratinocytes (KC) produce biologically relevant amounts of IL-7 and that this cytokine promotes the survival and growth of DETC in vitro. In the present study, we examined whether IL-7 also upregulates TCR/CD3 expression by DETC. The long-term cultured DETC line 7-17, which constitutively expresses a TCR and CD3, was incubated for 48 hr in the presence rIL-7. As judged by FACS, cell surface expression of the $\gamma\delta$ TCR, Vγ3 and CD3 increased by 2-fold, compared with IL-7(-) controls. Dose dependency experiments demonstrated optimal upregulation to occur at 5 ng/ml IL-7. Significant upregulation was detectable as early as 3 hr after IL-7 introduction. Similar upregulation was also observed in cells treated with rIL-2. By contrast other cytokines known to be secreted by KC (IL-1α, IL-6, IL-8, IL-10, GM-CSF or TNFα) failed to alter TCR/CD3 expression. To study this regulation more closely, 7-17 DETC were labeled with ^{35}S-methionine during incubation with IL-7 and then subjected to immunoprecipitation; cells treated with IL-7 exhibited significantly higher levels of new CD3 synthesis than did IL-7(-) controls. Moreover, Northern blot analyses revealed a two-fold increase in TCR γ-chain mRNA within 16hr after stimulation with IL-7. Thus, we propose that KC-derived IL-7 promotes not only the expansion, but also the phenotypic maturation of $\gamma\delta$ T cells within the epidermal microenvironment.

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EXPRESSION OF CYTOKINE mRNA BY MURINE FIBROBLAST-LIKE CUTANEOUS STROMAL CELL (CSC) LINES WHICH SUPPORT THE GROWTH OF CUTANEOUS $\gamma\delta$ TCR$^+$ T CELLS. M. Deguchi, S. Aiba, S. Nakagawa, H. Tagami, Department of Dermatology, Tohoku University, Sendai, Japan.

Recently we have published that fibroblast-like CSC line, DFB-1, and clone, 12E2, which we established from the skin of BALB/c mouse ears, can support the growth of murine cutaneous $\gamma\delta$ TcR$^+$ T cells with IL-2. This growth enhancement is partially mediated by soluble factors. The purpose of this study was to examine which kinds of cytokines were produced by these CSCs and to compare them with the cytokines produced by keratinocyte cell line, PAM212. Total RNA from 12E2, DFB-1, and PAM212 was extracted and was reverse-transcribed. PCR amplification was performed for 30 cycles with the specific primers (CLONTECH) and Southern blot analysis was performed by the standard procedure. The DNA probes were made by PCR of the standard DNA (CLONTECH) in the presence of biotinylated dUTP. The validity of the sequences of these probes was confirmed by mapping study using restriction enzymes. Among 10 cytokine mRNA, such as IL-1 α, β, IL-3 to IL-7, GM-CSF, TNF α, and IFN γ, both DFB-1 and PAM212 produced IL-1 α, β, IL-3, 5, 6, 7, GM-CSF, and TNF α mRNA, while 12E2 produced only IL-5, 6, 7, GM-CSF, and TNF α mRNA. On the concept of Skin-Associated Lymphoid Tissue, the cytokines produced by keratinocytes have been extensively studied. In contrast, the immunological function of dermal fibroblasts have been ignored. In this study, we could not detect unique cytokines only produced by CSCs, which might be responsible for their ability to support the growth of cutaneous $\gamma\delta$ TcR$^+$ T cells. However, these data revealed that CSCs, probably fibroblasts, could produce the cytokines similar to those produced by keratinocytes, which suggested that dermal fibroblasts also played the important immunological roles.

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Th2 CYTOKINE PATTERN IN CUTANEOUS T CELL LYMPHOMA CORRELATES WITH PRESENCE OF MALIGNANT T CELL CLONE: IMPLICATIONS FOR CTCL ETIOLOGY, PROGRESSION, AND THERAPY. BR Vowels, M Cassin, SR Lessin, BM Benoit, and AH Rook. Department of Dermatology, University of Pennsylvania, School of Medicine, Philadelphia, PA, USA

We have previously demonstrated that peripheral blood mononuclear cells (PBMC) from Sezary Syndrome (SzS) patients have a Th2 cell cytokine pattern. In this study, we extend these observations to correlate the presence of a Th2 cytokine pattern with a malignant T cell clone in 1) different stages of cutaneous involvement and 2) treatment with biological response modifiers (BRM). Skin biopsies were obtained from 14 CTCL patients at various disease stages (3 patch(PA), 3 plaque (PL), 8 tumor (TU)), 3 patients with parapsoriasis (PPs, a CTCL-associated skin disease), 4 patients with inflammatory dermatoses (ID), and 13 normal controls. Total RNA was extracted, reverse-transcribed and PCR-amplified for IL-2, IL-4, IL-5, and IFN-γ. Greater than 90% of skin biopsies from patients and normal controls expressed mRNA for IL-2 and IFN-γ. IL-4 mRNA was demonstrated in skin biopsies from 7/8 TU stage, 1/3 PL stage, 0/3 PA stage and 0/3 PPs patients. IL-5 mRNA was demonstrated to be present in 7/8 TU stage, 2/3 PL stage, 1/3 PA stage and 1/3 PPs patients. In addition, biopsies taken from involved and uninvolved areas of the same patient showed Th2 cytokines only in the involved areas. None of the normal controls, or ID patients expressed mRNA for IL-4 or IL-5. These data further support a Th2 cell phenotype for the malignant clone because 1) Th2 cytokine mRNA pattern of PBMC from SzS is also present in the skin of patients with earlier stage disease; and 2) there is a direct correlation between the stage of disease, number of malignant T cells and Th2 cytokine expression. These data suggest that the factors responsible for differentiation to a Th2 cell may contribute to the etiology of CTCL and that biological response modifiers that inhibit Th2 cells may be therapeutic for CTCL.

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IN-SITU EXPRESSION OF THE TH1-LIKE CYTOKINE INTERFERON γ (IFNγ) IN LESIONAL SKIN IS LINKED TO THE CLINICAL SEVERITY OF ATOPIC DERMATITIS (AD). Markus Grewe, Karin Gyufko, Rüdiger Block, Erwin Schöpf, and Jean Krutmann, Photodermatology Section, Department of Dermatology, University of Freiburg, Freiburg, Germany.

The inflammatory infiltrate present in lesional skin of patients with AD is dominated by CD4$^+$-T-cells. CD4$^+$-T-cells may be grouped into Th1-like cells, which preferably produce IFNγ, and Th2-like cells, which preferably secrete IL-4. To investigate the relevance of the in situ expression of Th1- and Th-2-like cytokines for the clinical course of AD, in the present study, RNA was extracted from biopsies obtained from lichenified atopic skin before and after therapy, and mRNA signals for IFNγ and IL-4 were analyzed by semiquantitative RT-PCR. As compared to normal skin, enhanced IFNγ mRNA signals were observed in lesional atopic skin in 12 out of 14 patients before therapy. High-Dose-UVA1 irradiation (n=9) or topical glucocorticoids (n=2) induced a significant clinical improvement, and in biopsies obtained from these patients after therapy, by employing identical PCR conditions, no IFNγ-mRNA specific signals could be detected. In contrast to IFNγ mRNA, expression of IL-4 mRNA could be detected in one third of these patients before as well as after therapy. The potential relevance of the in situ expression of IFNγ mRNA for the clinical course of AD is further suggested from results obtained in UVAB irradiated patients. UVAB therapy (n=3) failed to induce a significant clinical improvement, and in biopsies obtained from these patients, significant levels of IFNγ mRNA were detected both before and after therapy. These studies indicate that IFNγ expression in lesional atopic skin is linked to the clinical severity of AD.

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STAPHYLOCOCCAL ENTEROTOXIN B INDUCES *IN VITRO* IL-4 PRODUCTION AND IGE SYNTHESIS IN PATIENTS WITH ATOPIC ECZEMA. Karsten Neuber, Katharina Steinrücke and Johannes Ring, Department of Dermatology, University Hospital Eppendorf, University of Hamburg, Germany

The skin of patients with atopic eczema (AE) is often colonized with *Staphylococcus aureus*. Over 50% of *S.aureus* strains secrete exotoxins (e.g. staphylococcal enterotoxin B [SEB] or toxic shock syndrom toxin-1 [TSST-1]). These toxins are of special immunological interest because they are potent stimulators of T-cells and monocytes. About 60% of patients with AE demonstrate specific IgE antibodies to SEB or TSST-1. In this study the influence of SEB on the IgE synthesis and interleukin secretion (IL-2, IL-2R, IFNτ and IL-4) of peripheral blood mononuclear cells (PBMC) of patients with AE (n=5) *in vitro* was investigated. Healthy non-atopic volunteers served as controls. Incubation of PBMC with SEB (1 μg/1 x 10^6 cells) resulted in a significantly enhanced IgE-synthesis and IL-4 production in patients with AE, whereas there was no effect in normals. In contrast, SEB induced a markedly increased IL-2, IL-2R and IFNτ secretion in PBMC from healthy volunteers, while PBMC from atopics showed a significantly weaker production of these cytokines. The data support the assumption that staphylococcal skin colonization might act via SEB as amplifier of allergic IgE-mediated inflammatory reactions in patients with AE.

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CYTOKINE PRODUCTION OF PERIPHERAL BLOOD MONONUCLEAR CELLS FROM A PATIENT WITH GOLD CONTACT ALLERGY. Tetsuya Koga, Shuhei Imayama, and Yoshiaki Hori, Department of Dermatology, Kyushu University Faculty of Medicine, Fukuoka, Japan

Allergic contact dermatitis is a skin manifestation of delayed-type hypersensitivity (DTH) reaction mediated by T lymphocytes. When an antigen is applied to the skin, specifically sensitized T lymphocytes proliferate and release certain cytokines, thus producting inflammation characterized histologically by spongiosis of the epidermis and infilrtate of mononuclear cells, largely of T lymphocytes. Cytokines secreted by these lymphocytes include IL-2, IFN-γ and GM-CSF, known as the important factors in the development of the DTH reaction. In this context, we measured cytokine production in response to stimulation with gold in peripheral blood mononuclear cells (PBMC) obtained from a patient with a contact allergy to gold, and evaluated usefulness of the cytokine assay as the diagnosis and/or investigation of allergic contact dermatitis. IFN-γ and IL-2 activities in the culture supernatant were determined using RIA test kit. GM-CSF activity was measured by a solid-phase ELISA. IFN-γ, IL-2 and GM-CSF were detected in the culture supernatant from the patient's PBMC, but not in the supernatant from PBMC obtained from a non-allergic healthy donor. These findings indicate that a patient with gold contact dermatitis has gold-specific T-lymphocytes circulating in the blood and is able to produce IFN-γ, IL-2 and GM-CSF. Since antigen-induced cytokine release is a crucial step in a DTH reaction, assays that measure antigen-induced cytokine production may be suitable as an alternative test for the diagnosis and/or investigation of allergic contact dermatitis.

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CYTOKINE PROFILE IN CHEMICALLY INJURED SKIN. Tsuyoshi Matsunaga, Hiroo Yokozeki, Ichiro Katayama and Kiyoshi Nishioka, Department of Dermatology, Tokyo Medical and Dental University Faculty of Medicine, Tokyo, Japan

To clarify the mechanism of inflammatory response in chemically injured skin, we investigated the cytokine production and cell adhesion molecule expression of chemically stimulated organ cultured skin by immunohistochemistry and reverse transcriptase-polymerase chain reaction (RT-PCR) technique. Various chemical substances were applied on sliced skin specimen or added in culture medium at the initiation of the organ culture. Each skin specimen was recovered at various time and processed for immunohistochemical or RT-PCR analysis. Cytokine contents in culture supernatant were assessed by ELISA kit. IL-1β was induced in whole epidermis at early stage (3 hr after painting) by both sensitizer (DNCB) and non-sensitizer (methyl salicylate) followed by TNF-α expression in a similar manner to IL-1β at 12 hours. DNBS (tolerogen), retinoic acid (RA) or vehicle control (acetone, ethanol) induced rather weak expression of these cytokines similar to non-stimulated culture. In contrast to cytokine profile, ICAM-1 was strongly expressed in basal layer at 3 hours and in spinous layer at 12 hours by RA. Other chemicals also induced weak ICAM-1 expression in basal layer at 12 or 24 hours. These results suggest that cytokine production and cell adhesion molecule expression might be controlled by different mechanism in chemically stimulated keratinocytes and no clear distinction was observed between sensitizing and non-sensitizing agents.

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CROSS-REGULATORY ROLES OF IL-10 AND IL-12 IN LEPROSY SKIN LESIONS. Peter A. Sieling, Thomas H. Rea and Robert L. Modlin, Division of Dermatology, UCLA and USC School of Medicine, Los Angeles, California.

Leprosy provides a model to investigate the role of cytokines in the immune response to infection in skin. Th1 cytokines, IL-2 and IFN-γ, are present in tuberculoid lesions characterized by effective cell-mediated immunity (CMI) against M. leprae. Th2 cytokines, IL-4 and IL-10, are present in lepromatous lesions, in which CMI is absent and the infection progresses. Here we investigated the effects of IL-10 and IL-12 on CMI in leprosy. IL-12 mRNA, as determined by PCR, was more strongly expressed in tuberculoid than lepromatous lesions (relative cpm 58±8 vs. 34±6, $p<0.02$). Neutralizing anti-IL12 antibodies blocked (up to 80%) T-cell responses to M. leprae in tuberculoid patients in vitro. Although T-cell responses to M. leprae in lepromatous patients was weak, rIL-12 increased these responses by 2-4 fold. Most strikingly, rIL-12 plus anti-IL10 synergistically restored T-cell responsiveness to M. leprae in lepromatous patients in vitro. Anti-IL10 also augmented in vitro production of IFN-γ, TNF-α and GM-CSF, cytokines with antimycobacterial properties. The data suggest that the battle between IL-10 and IL-12 determines the level of T-cell responsiveness to infection in skin.

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STRUCTURAL, FUNCTIONAL, AND LIPID METABOLIC CHANGES IN THE AGED EPIDERMAL PERMEABILITY BARRIER. Ruby Ghadially, Barbara E. Brown, Sandy M. Sequeira-Martin, Peter M. Elias. Dermatology VAMC, and Department of Dermatology, University of California, San Francisco, Ca., USA.

Aged epidermis displays altered drug permeability, increased susceptibility to irritant contact dermatitis, and often severe xerosis. Yet, little is known about the functional, structural and lipid biochemical basis of epidermal aging. In order to compare epidermal barrier function in aged (>75 years) vs. young (20-30 years) human subjects we measured basal transepidermal water loss (TEWL), barrier integrity (resistance to either sequential tape stripping or acetone wipes), and barrier repair after abrogation. Lamellar bilayers and the lamellar body secretory system were assessed in aged and young human epidermis, post-fixed in 0.2% ruthenium tetroxide or 1% osmium tetroxide with 0.5% $K_4Fe(CN)_6$, and processed for electron microscopy. Stratum corneum lipid content and epidermal lipid synthesis from $^3H_20$ incorporation was also compared in aged vs. young hairless mice (hrNhr).

Baseline TEWL in the aged was decreased. However, the epidermal permeability barrier was more readily perturbed with either acetone or tape stripping (10-15 strippings in aged subjects vs. 25-40 strippings in young subjects to acheive a TEWL of greater than 18 mg/m^2/h). Moreover, the barrier recovered more slowly in aged subjects. Young subjects exhibited 50% and 80% recovery at 24 and 72 hours respectively, while the aged showed only 15% recovery at 24 hrs followed by a further lag over the next 6 days. Ultrastructurally there was disorganization and paucity of lamellar bilayers in the stratum corneum and a decrease in lamellar body contents. Total lipid content was decreased (~ 30%) in aged mice, but lipid species distribution was unchanged. Although lipid synthesis was up-regulated after barrier perturbation in the aged as in the young, both basal and post-perturbation synthesis rates were lower. Thus while barrier function is normal under basal conditions, barrier reserve is diminished and repair is slower, resulting in increased susceptibility to exogenous insult.

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DEFICIENCY OF ß-GLUCOCEREBROSIDASE RESULTS IN ABNORMAL STRATUM CORNEUM LAMELLAR BILAYER MATURATION. Walter M. Holleran, Ellen Sidransky, Gopinathan K. Menon, Peter M. Elias, Edward I. Ginns. Dept. Dermatology, University of Calif. & Derm. Serv., V. A. Med. Ctr., San Francisco, CA; and Section on Molecular Neurogenetics, National Institutes of Mental Health, Bethesda, MD, USA

The hydrolysis of glucosylceramide (GlcCer) to ceramide (Cer) by ß-glucocerebrosidase (GCase) appears responsible for the increased Cer and decreased GlcCer in stratum corneum (SC). Inhibition of murine epidermal GCase with bromoconduritol B epoxide (BrCBE) leads to abnormal permeability barrier function, increased GlcCer in SC, and altered SC lamellar bilayer structures (J. Clin. Invest. 91:1656, 1993). To ascertain further whether conversion of GlcCer to Cer is a prerequisite for barrier function, we examined SC structure both in GCase-deficient transgenic mice, and in severely GCase-deficient Gaucher type 2 patients. Gaucher mice had <4% of normal GCase activity in tail samples, while carriers and normals had 78±7 and 138±3 nmol/mg/hr, respectively (genotypes confirmed by Southern analysis). Both epidermis and SC of Gaucher mice demonstrated 5-to-10-fold elevated GlcCer with diminished Cer when compared to heterozygous and normal littermates. Using RuO_4 to visualize extracellular membrane domains, lamellar bilayer structures were normal in both carrier and normal littermates. In contrast, Gaucher mice had extensive alterations in lamellar bilayer structures, including persistence of incompletely processed bilayers into the outer SC, and focal areas of abnormal lipid structures with whorl-like patterns. No abnormalities were observed within lamellar bodies, and a normal pattern of lamellar body secretion was present at the stratum granulosum/SC interface. Similar ultrastructural findings also were observed in samples from two severely GCase-deficient Gaucher type 2 patients. Since analogous morphologic changes occur following BrCBE-inhibition of epidermal GCase, these results further demonstrate the importance of GlcCer to Cer hydrolysis for maturation of intercellular lamellar bilayers required for the epidermal permeability barrier of terrestrial mammals.

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BARRIER FORMATION IN AIR-EXPOSED KERATINOCYTE CULTURE: ULTRA STRUCTURE AND LIPID COMPOSITION. Manigé Fartasch*, Maja Ponec, *Dept. of Dermatology, University of Erlangen, Germany, Dept. of Dermatology, University Hospital Leiden, The Netherlands.

The ability of air-exposed human keratinocyte cultures to generate a competent barrier, i.e. the stratum corneum (SC), enables to use them as suitable models for the study of water barrier in vitro and skin toxicity studies. The intercellular epidermal lipids (EL) of the SC represent the vital factor for the permeability barrier of the skin. Ultrastructurally, we investigated the barrier development and spatial organization of the multilamellar EL of two different air-exposed keratinocyte cultures (1. human keratinocytes grown on deepidermized epidermis (DED) with medium A, 2. keratinocytes grown on dermal equivalent with medium B = living skin equivalent (LSE) of Organogenesis Inc.) with a new staining protocol (0.5% ruthenium tetroxide (RuO_4)/0.25 % $KFe(CN)_6$ -fixation). Additionally the influence of two medium (A and B) on the differentiation of the keratinocytes grown on DED (culture 1) were studied. Comparison with SC of excised human epidermis showed that the process of post-secretory extracellular processing of the lamellar body (LB)-derived lipids into the lamellar lipid bilayers at the str. granulosum (SG)/ SC layer interface seems to be disturbed in both culture systems. Only in culture 1 the formation of broad LB lipid sheets could be observed focally. In LSE (culture 2) no regularly lamellar arrangement of EL was found. In both cultures at all levels of SC concentric arranged lipid accumulation dilatating the intercellular spaces were depicted. By using the medium B the culture system 1 was able to form multilamellar lipid bilayers with basic unit repetition pattern seen in normal human SC. By applying the RuO_4 staining technique the barrier structures of different air-exposed keratinocyte cultures could be analyzed in detail and even visualized improvements of barrier structures by the modification of culture medium composition.

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LORICRIN IMMUNOREACTIVITY IN HUMAN SKIN: LOCALIZATION TO SPECIFIC GRANULES (L-GRANULES) IN ACROSYRINGIA. Akemi Ishida-Yamamoto[1,2], Daniel Hohl[3], Dennis R. Roop[4], Hajime Iizuka[1] and Robin A. J. Eady[2], [1]Department of Dermatology, Asahikawa Medical College, Asahikawa, Japan, [2]Department of Cell Pathology, St John's Institute of Dermatology, St Thomas's Hospital, London, UK, [3]Service de Dermato-venereologie, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland, [4]Department of Cell Biology and Dermatology, Baylor College of Medicine, Houston, Texas

Loricrin is a major component of the cornified cell envelope and is also expressed in the granular layer of human epidermis. In newborn mouse epidermis, loricrin accumulates in small round granules (L-granules) in the granular layer before being incorporated into the cornified cell envelope, but the expression of L-granules has not yet been demonstrated in human skin. In the present study we used postembedding immunoelectron microscopy to examine loricrin expression in normal human skin. We observed diffuse loricrin staining in the uppermost granular cell layer in interappendageal epidermis, that was not associated with any granular structures. In the cornified cells, most of the labeling was on the inner face of cornified cell envelopes. By contrast, in the upper segment of acrosyringia there were small granules that specificaly labeled for loricrin. In the peripheral ductal cells, L-granules with a highly osmiophilic electron density appeared in the nucleus as well as in the cytoplasm. In the luminal ductal cells, L-granules were round or oval, less-electron dense and larger than the peripheral cell L-granules, and present only in the cytoplasm. Some of the peripheral cell L-granules and most of the luminal cell L-granules formed composite granules with filaggrin immunoreactive granules. These results suggest that in human interappendageal epidermis, loricrin might be rapidly incorporated into the cornified cell envelope without prior accumulation in any type of granule. By contrast, in acrosyringia loricrin accumulates in granules perhaps reflecting increased synthesis or slower processing.

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ISOLATION AND CHARACTERIZATION OF A MOUSE HAIR FOLLICLE-SPECIFIC GENE. N. Huh, C. Konishi, M. Kashiwagi, Q.Lei, Y. Kohno, and T. Kuroki, Dept. Cancer Cell Res., Inst. Med. Science, University of Tokyo, Tokyo, Japan

We have isolated a clone tentatively termed hacl-1 from cDNA library of ICR mouse skin. Hacl-1 is expressed specifically in skin, and its mRNA levels were correlated with the active state of hair follicles in developmental and regenerative processes of hair. The size of mRNA is ~1 kb. Genomic clones were isolated using hacl-1 cDNA as a probe. The cDNA was completely co-linear with the genomic clone, indicating that hacl-1 gene is composed of only one exon. Hacl-1 gene has an ORF of 600 bp. The deduced amino acid sequence showed six direct repeats of decapeptide at C-terminal side. In situ hybridization using 3' untranslated region of hacl-1 cDNA as a probe demonstrated that hacl-1 is expressed specifically in the cortical cells of hair shaft at certain stage of differentiation. No other components of hair follicles nor epidermal cells showed positive signal.

Hacl-1 showed some structural similarity with mouse hair-specific IFAP (intermediate filament associated protein)-type keratin genes thus far reported, ultra-high-sulfur keratin (McNab et al., 1989) and serine-rich ultra high sulfur keratin-1 and -2 (Wood et al., 1990). However, cysteine or serine content of hacl-1 is not remarkably high. These lines of evidence suggest that hacl-1 represent a novel hair specific IFAP type-keratin.

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LOCALISATION AND ACTIVITY OF NITRIC OXIDE SYNTHASE DURING THE MURINE HAIR CYCLE Dippel E, Schönfelder G, Czarnetzki BM and R Paus Dept. of Dermatology, University Hospital R. Virchow, Freie Universität Berlin, Germany

The free diffusible radical, nitric oxide (NO), is generated by nitric oxide synthase (NOS). The pleiotropic, bioregulatory molecule, NO, regulates e.g. the vascular tone, is a major neuro- transmitter and is involved in macrophage-mediated cytotoxicity. NOS exhibits NADPH diaphorase (NADPH-d) activity that can be demonstrated histochemically. To study whether NOS is present in mammalian skin and whether any such activity may be developmentally regulated, we have examined cryosections of C 57 BL-6 mouse skin in various well-defined hair cycle stages. The histochemical analysis of NOS activity (NADPH-d) was complemented by immunohistology using two specific rabbit antisera against porcine and rat cerebellar NOS. NOS antigen and activity were seen in epidermis, outer root sheath, distal hair bulb, sweat glands and subcutaneous smooth muscle layer. This is the first demonstration of NOS antigen and activity in murine skin. Most obvious changes of NADPH-d activity during the depilation-induced murine hair cycle were seen in the dermal papilla between early and late anagen follicles. All cells showing NOS antigen immunoreactivity also demonstrated NADPH-d activity. Our findings suggest that normal murine skin has multiple cellular sources capable of synthesizing NO and that this capacity is developmentally regulated.

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LOCALIZATION OF ALKALINE PHOSPHATASE IN ECCRINE AND APOCRINE SWEAT GLANDS. Kenji Saga, Yousuke Morimoto, and Makoto Takahashi, Department of Dermatology, Sapporo Medical University, Sapporo, Japan

Alkaline phosphatase (AP) is a group of enzymes that are membrane-bound glycoproteins. AP catalyzes the hydrolysis of inorganic and organic monophosphate esters at alkaline pH. Although this enzyme is widely distributed in human tissues, we don't fully understand its physiological function. The presence of AP in the kidney, liver, and intestine has suggested that the enzyme might participate in membrane transport. The localization of AP in human sweat glands was poorly understood. Therefore, we tried to elucidate the localization of AP in human eccrine and apocrine sweat glands. Light and electron microscopic enzyme cytochemistries were employed for the localization of AP in human sweat glands. For demonstration of AP at the light microscopic level, frozen sections were cut from human skin and fixed in cold acetone. Sections were incubated in the reaction medium that included naphthol AS-MX phosphate and Fast Red RC salt. For demonstration of AP at the electron microscopic level, small pieces of skin were fixed in the aldehyde fixative, then sweat glands were isolated under a stereomicroscope using tweezers. Isolated sweat glands were incubated in the reaction medium that contained β-glycerophosphate and lead citrate. Thereafter sweat glands were embedded in Epon 812 and thin sections were observed. Light microscopic enzyme cytochemistry demonstrated strong reaction in the intercellular canaliculi of human eccrine sweat glands, while secretory cells of apocrine sweat gland did not show any reaction. Myoepithelial cells of eccrine and apocrine sweat glands showed weak reaction. Electron microscopic enzyme cytochemistry demonstrated the localization of AP on the cell membrane of intercellular canaliculi of eccrine sweat glands. The luminal cell membrane of eccrine secretory cells that is in continuity with intercellular canaliculi was devoid of this reaction. These data suggest that the intercellular canaliculi are not simply an extension of the luminal cell membrane of eccrine secretory cells. Intercellular canaliculi are a differentiated structure that may play a role in the production of primary sweat.

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AMOUNT OF SECRETORY IMMUNOGLOBULIN-A DIFFERES WITH SKIN REGION BUT MAINTAINS ITS LEVEL REGARDLESS OF SWEATING. Shuhei Imayama, Yuji Shimozono, Atsumichi Urabe, Yoshiaki Hori, Shigeo Ohta, and Keishi Yoneyama, Department of Dermatology (SI, AU, YH), Faculty of Medicine, Kyushu University, Fukuoka; Hisamitsu Pharmaceuticals (YS, SO, KY), Tosu, Japan

We developed a simple method for measuring the amount of the secretory form of immunoglobulin A (sIgA) present in sweat. A small disk (10x10mm) made of cellulose membrane was attached to the skin surface for periods of 1 to 24 hours. SIgA was absorbed to the membrane and accumulated during the period of application. An enzyme immunoassay using anti-IgA and anti-secretory component (SC) antibodies revealed dots on the disk which corresponded to the eccrine excretory ducts. A densitograph was used to determine the number and density of the dots, thus obtaining the amount of sIgA excreted to the surface of the skin (per mm^2). The amount of skin sIgA excreted in mm^2/day by 50 healthy subjects differed inter-individually as well as intra-individually; it varied according to region of the skin, and its distribution roughly reflected that of the sweat ducts. SIgA excretion was maintained at a certain level regardless of the increased sweating produced by either heat or exercise, which raised the output of sweat by 3- to 15-fold. Immunohistochemical studies revealed that fewer glandular cells expressed SC in their cytoplasm as the amount of sIgA decreased. Such an independence of the sIgA excretion from sweat excretion may be necessary to local immune defenses.

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PROTEIN KINASE C-δ BUT NOT PROTEIN KINASE C-β IS THE PREDOMINATE ISOENZYME EXPRESSED IN LANGERHANS' CELLS IN SITU. Lutz Hegemann, Renate Knaup, Andrea Wevers, Klaus Wolff, and Gustav Mahrle, Departments of Dermatology, University of Köln, Köln, Germany, and University of Vienna, Vienna, Austria.

There is increasing evidence indicating that the isoenzymes of the protein kinase C (PKC) family play discrete roles in epidermal cell function. In earlier studies, we have demonstrated that the pattern of isoenzyme expression changes during keratinocyte proliferation and differentiation in cultured cells. It has previously been reported that PKC-β is expressed in Langerhans' cells *in situ*. Therefore, we investigated the expression of PKC isoenzymes in normal human skin. PKC-β, as well as PKC-α, were found to be exclusively expressed in basal keratinocytes. In the case of PKC-β, this finding was confirmed by *in situ* hybridization, which revealed the absence of mRNA encoding for PKC-β in suprabasal layers of the epidermis. In contrast, PKC-δ was homogenously expressed in all keratinocyte layers but was found to be highly expressed in dendritic cells. Even at lower concentrations of the antibody, at which the keratinocyte staining was almost abolished, the dendritic cells remained intensely stained. Therefore, we investigated the expression of PKC-δ in these cells in more detail, applying double staining for PKC-δ and OKT 6. We found that PKC-δ is only expressed in OKT 6-positive cells. In summary, the present data demonstrate that PKC-δ rather than PKC-β is expressed in human Langerhans' cells. The functional role of this highly expressed isoenzyme in Langerhans' cells is under current investigation.

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COMPUTER ASSISTED THREE-DIMENSIONAL RECONSTRUCTIONS OF DERMAL DENDROCYTES. Hirohiko Sueki, Brett Telegan and George F. Murphy, Dept. of Dermatology, Univ. of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA.

The purpose of this study was to characterize the three-dimensional (3D) structure of dermal dendrocytes (DDs), and to clarify the spatial relationships between DDs and mast cells, macrophages and nerves. Normal human adult skin (breast, n=2) were routinely processed for electron microscopy (EM). Serial sections (about 50/ data set) were collected at 80 nm thickness intervals traversing about 4 microns of tissue. Grids showing the same cells were photographed by EM at a magnification of 4000x noting the section position in the serial set, thereby preserving the depth dimension. 8x10 inches micrographs were enlarged to 11x17 inches by photocopy yielding a final magnification of 7500x and assigned layer numbers based on section position. Cell outlines were digitized into the reconstruction program at appropriate layers and aligned (J Electron Microscopy Technique 6: 207, 1987). Having the entire 3D data set in the computer allowed viewing from various complimentary angles to investigate spatial relationships not perceived from individual electron micrographs.

Thin, elongated cytoplasmic "dendrites" of DDs in two-dimensional (2D) micrographs proved to be thin, membrane-bound flaps in 3D reconstruction. These flaps appeared as slender, conical dendrites only in single 80 nm thin sections where they were represented as cross-sectional profiles. For DDs concentrated about superficial vessels (perivascular dendrocytes; PVD), the flaps enshrouded the vessel wall, and for DDs directly beneath the epidermis (subepidermal dendrocytes; SED), these flaps were parallel to the dermal-epidermal junction. In conventional ultrathin sections, 20-40% of PVDs and occasional SEDs were closely associated with mast cells. When viewed by computer assisted 3D reconstruction, membrane flaps of DDs consistently shrouded mast cell membranes for 50-90% of their perimeter, like a ball (mast cell) in a baseball glove (DD). Occasional DDs surrounded non-myelinated nerves in superficial dermis. Dermal dendrocytes, which surrounded both nerves and mast cells simultaneously, were also observed. Membrane flaps also enabled DDs to display extensive areas to plasma membranes of adjacent monocyte/macrophages.

These findings indicate that DDs are non-dendritic cells that are spatially related to mast cells, monocyte/ macrophages, microvessels and nerves by their membranous flaps. This suggests the need for further study of functional interactions between these cells.

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BULLOUS CONGENITAL ICHTHYOSIFORM ERYTHRODERMA OF BROCQ CAUSED BY BOTH DISRUPTIVE AND HIGHLY CONSERVATIVE AMINO ACID SUBSTITUTIONS IN THE 1A DOMAIN OF KERATINS 1 AND 10.

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Bullous congenital ichthyosiform erythroderma (BCIE) is a human autosomal dominant skin disorder characterised by severe epidermolytic hyperkeratosis. Recently, point mutations in either of the suprabasal keratins 1 and 10 have been shown to cause this disease. The mutations reported to date are predicted to be highly disruptive. Here, we report 4 mutations in the α-helical 1A domain of K1 and K10. A point mutation in K1 and another in K10 produce proline substitutions, which are shown by protein structural predictions to be highly detrimental to α-helix formation. In addition, we report highly conservative amino acid substitutions in K1 and K10 which also produce the disorder. Interestingly, the phenotype is very severe in all 4 patients, indicating that even subtle changes in this highly conserved region of keratin intermediate filaments cannot be tolerated *in vivo*.

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ULTRASTRUCTURAL LOCALIZATION OF BINDING SITES FOR LINEAR IgA BULLOUS DERMATOSIS (LABD) ANTIBODIES.

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Linear IgA bullous dermatosis (LABD) is characterized by the presence of linear IgA deposits in the cutaneous basement membrane zone. At least two patterns of deposition can be detected by direct immuoelectron microscopy (IEM): in the lamina lucida (LL) and in the sub- lamina densa (LD) zone. A particular "mirror image" pattern combining deposits on both sides of the LD has been also described.

In the present indirect IEM study, sera of 26 patients with LABD were used for precise localization of the target antigens in two substrates: normal human skin and monkey esophagus. The sera were selected using indirect immunofluorescence, on the basis of their reactivity with salt-split skin: 24 sera bound to the roof of the bulla, 1 to the roof and the floor, and 2 to the floor alone. On IEM, the latter two sera gave a predominant sub-LD immunoperoxidase labeling, however, a concomitant linear staining in the LL was also observed. Out of the remaining 24 sera, 13 stained hemidesmosomes (50%), 9 labeled the LL (34.6%), and two were unreactive (on either substrate). Most sera reacted with monkey esophagus, whereas several ones were negative on human skin. For these reacting with both tissues, the labeling pattern was always reproducible. A LABD serum recognizing the 97 kD protein (J.J. Zone et al., J. Clin. Invest. 85: 812-820,1990) labeled the LL. Control sera containing IgG autoantibodies against the 180 kD and 230 kD bullous pemphigoid and the 290 kD epidermolysis bullosa acquisita antigens reacted with the LL, hemidesmosomes, and sub-LD zone, respectively. No relationship between the clinical picture and the observed staining pattern of LABD sera could be found.

Our results confirm the heterogeneous nature of LABD but do not support the concept of clinical significance of any particular localization of binding sites for LABD autoantibodies within the basement membrane zone.

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ANTI-CD34 IMMUNOSTAINING DISCRIMINATES BETWEEN SCAR AND RESIDUAL DERMATOFIBROSARCOMA PROTUBERANS.

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Dermatofibrosarcoma protuberans (DFSP) is a cutaneous malignant mesenchymal tumor, classically included among fibrohistiocytic neoplasms. It usually presents in middle-aged patients as a large tumor of truncal skin. After biopsy, the suggested treatment is wide re-excision, with free margins. The histologic features of DFSP may be very similar to those observed in a recent surgical scar, and sometimes it is very difficult to determine on histologic grounds alone if there is any residual DFSP present at the margins.

CD34 is an antigen present in bone-marrow stem cells, endothelial cells, and dermal dendritic cells. Lately, DFSP has also been shown to express CD34; no studies have been reported on CD34 expression in recent scars. This study was performed to test whether CD34 expression can discriminate between DFSPs and scars. Anti-CD34 immunostaining (anti-HPCA-1, Becton-Dickinson) was performed on 10 DFSPs and 10 scars from re-excision of other skin tumors (melanoma or basal cell carcinoma). All 10 DFSPs had strong, diffuse cytoplasmic CD34 staining of the tumor cells. In contrast, all 10 scars were negative; only blood vessels and rare dendritic cells, which did not form well-defined fascicles, were CD34 positive.

Anti-CD34 immunostaining discriminates between DFSP and scar tissue, and therefore may be very helpful in determining the presence of residual DFSP in the surgical scar of re-excision specimens.

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SYSTEMIC SUPPRESSION OF HYPERSENSITIVITY TYPE GRANULOMA BY HIGH DOSE UVB RADIATION.

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Granulomatous inflammation is broadly classified as foreign body type- and hypersensitivity type- granulomas. To study the influence of high dose UVB radiation on granuloma formation, pulmonary granulomas were induced by artificial microparticles. Exposure of mice to 5 J/cm² UVB radiation did not affect the formation of foreign body type granuloma in lungs induced by intratracheal injection of dextran beads. In contrast, UVB radiation significantly suppressed pulmonary hypersensitivity type granuloma induced by intratracheal injection of keyhole lympet hemocyanin (KLH)-coupled agarose beads, when mice were irradiated before the sensitization with KLH. The suppression was transfered into syngeneic recipient mice by spleen T lymphocytes from mice exposed to UVB radiation and then sensitized with KLH. This suppression was highly selective, because the spleen cells from UVB-irradiated and then KLH-sensitized mice did not affect the formation of bovine serum albumin (BSA)-coupled agarose bead-induced granulomas. Supernatants derived from UV-irradiated Pam 212 keratinocyte cell line were evaluated for the ability to induce the suppression of granuloma formation after intravenous injection to mice. Injection of supernatants derived from UVB-treated Pam 212 cells significantly blocked induction of hypersensitivity type granuloma, while those from UVA-irradiated or nonirradiated Pam 212 cell line did not suppress the granuloma formation. These data suggested that the mechanism of suppression of hypersensitivity type granuloma may be similar to that responsible for UVB-induced immunosuppression of delayed type hypersensitivity.

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INHIBITORY EFFECT OF MELANIN PIGMENT ON SENSITIZATION AND ELICITATION OF MURINE CONTACT PHOTOSENSITIVITY: MECHANISM OF LOW RESPONSIVENESS IN C57BL/10 MICE.

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Murine contact photosensitivity (CPS) to 3,3',4',5-tetrachlorosalicylanilide (TCSA) is genetically controlled mainly by the major histocompatibility complex. The H-$2^{b,d}$ haplotypes are closely associated with high responders, while mice with the H-2^{k} are non-responders. Irrespective of their H-2 haplotypes, the C57BL/10 (B10) background strains, including B10, B10.D2, B10.A, and B10.BR, possessing black fur color, were low or non-responders in CPS to TCSA. In B10 mice, however, high sensitivity responses were induced when subcutaneous inoculation of epidermal cells (EC) photomodified in vitro with TCSA was used for both immunization and challenge, suggesting that the epicutaneous route for induction and elicitation is defective in B10 background mice. F_1 mice obtained by crossing high-responder BALB/c and low-responder B10 mice, possessing agouti fur color, were non-responders of CPS. The magnitude of CPS in the F_2 mice derived from F_1(BALB/c X B10) siblings varied from low to high. When these F_2 mice were divided into 5 groups with regard to fur color, the magnitude of reaction was correlated with the fur color and there was inverse relationship between the magnitude of CPS and the amount of melanin pigment in earlobe EC. Furthermore, the in vivo formation of TCSA-EC photoadducts was negatively correlated to the melanin amount in earlobes. These observations suggested that the failure in CPS of the B10 background mice stems from inability of in vivo photocoupling of TCSA to EC, presumably due to absorption of ultraviolet radiation by melanin pigment.

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MECHANISMS IN THE UVB-INDUCED DEPLETION OF DENDRITIC EPIDERMAL T CELLS FROM MURINE SKIN.

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Dendritic epidermal T cells (DETC) are resident epithelial γδ T cells found in normal mouse skin. DETC have been depleted from epidermis experimentally by UVB irradiation, and the present study was conducted to identify mechanisms by which this occurs. UVB irradiation (100-600 J/m²) of the 7-17 DETC line decreased cell viabilities significantly, and it produced the following features of apoptotic cell death (programmed cell death): 1) DNA fragmentation into 200 bp units, 2) chromatin condensation, and 3) a requirement for macromolecular synthesis. It was thus concluded that UVB radiation causes apoptotic cell death in DETC. DETC apoptosis was prevented by IL-7, which is produced by keratinocytes (KC), and we therefore examined whether UVB exposure alters IL-7 mRNA expression. Northern blot analyses revealed that UVB radiation (100 J/m²) abolished IL-7 mRNA expression in Pam 212 KC. This effect was not attributable to cell death because TNFα mRNA was upregulated. These results gave rise to the hypothesis that a UVB-induced deficiency in IL-7 augments DETC apoptosis. To test this hypothesis, CBA mice were exposed in vivo to UVB (100 J/m² x 3 days), and rIL-7 (10 ng x 3 days) or PBS was injected locally after each exposure. Three days after the last exposure, skin was excised and epidermal sheets were examined for DETC densities by staining with anti-Thy-1 mAb. DETC densities were diminished in UVB-exposed skin (180 ± 20 cells/mm²) compared with unirradiated skin (340 ± 50 cells/mm²). Importantly, this UVB-induced DETC depletion was inhibited by IL-7 (350 ± 110 cells/mm²). IL-7 did not alter DETC in unirradiated mice. Thus, we conclude that UVB induces DETC depletion by at least two related mechanisms: triggering apoptosis in DETC and downregulating KC production of IL-7, which ordinarily would prevent apoptosis.

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QUANTITATIVE ANALYSIS OF HUMAN DERMAL CELL SUSPENSIONS AFTER UV INJURY: A SMALL DERMAL MONOCYTE/MACROPHAGE SUBSET UNDERGOES MARKED EXPANSION WHEREAS POTENT APC's OF LANGERHANS/DENDRITIC CELL LINEAGE ARE DEPLETED. L. Meunier, A. Gonzalez-Ramos, L. Oberhelman, C. Hammerberg, and K.D. Cooper. Immunodermatology Unit, Dept. of Dermatology, Univ. of Mich., Ann Arbor, MI., and Department of Dermatology, University of Montpellier, Montpellier, France.

Antigen-presenting, suppressor cell-inducing, $CD1a^-DR^+CD36^+$ macrophages appear in the epidermis 3 days following ultraviolet B radiation (UVB) exposure of human skin. To analyze APC kinetics in the dermis, we prepared dermal cell suspensions from human keratomes for triple marker flow cytometric analysis. Epidermal cell (EC) and dermal cell (DC) suspensions were obtained from non exposed (C-EC, C-DC) and UVB irradiated sites (3 days after 4 MED) (UV-EC, UV-DC) of 18 healthy human volunteers. Epidermal Langerhans cells (LC) ($CD1a^+$ $CD1c^+$ $CD11b^-$ $CD11c^{dim}$ Fc gamma RII^+ $CD36^-$ $CD45^+$) comprised 100% of DR^+ C-EC, but LC only comprised $7 \pm 3\%$ of HLA-DR^+ UV-EC. HLA-DR^+ C-DC from normal dermis were heterogeneous and contained LC-like dendritic APC ($CD1a^+CD1c^+$), dermal macrophage subsets ($CD1a^-$, $CD1c^-$), and non bone marrow-derived cells. Potent APC activity was limited to the $CD1c^+$ $CD1b^+$ LC-like dermal subset. After UV, the $CD1^+$ dermal cells, like epidermal LC, were depleted. However, UV exposure induced a 10-fold expansion (to 10-15% of UV-DC) of the dermal macrophage subset which is analogous to the macrophages which appear in UV-EC. In fact, the vast majority of the HLA-DR^+ UV-EC ($75 \pm 5\%$) were contained within a distinct non cycling subset that lacked the dendritic antigen presenting cell markers CD1a and CD1c, but which expressed the macrophage integrins CD11b and CD11c and also Fc gamma RII, CD36 and CD45. In conclusion, UV depletes dermal, as well as epidermal, cells of LC/dendritic APC lineage. The UV-induced epidermal macrophage appears to originate from a DR^+ $CD1a^-$ $CD1c^-$ $CD11b^+$ $CD36^+$ Fc gamma RII^+ population that undergoes a marked expansion in the dermis after UV injury.

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EVIDENCE THAT SUSCEPTIBILITY TO ULTRAVIOLET-B INITIATED SKIN DISEASES IN MAN MAY BE GENETICALLY DETERMINED. J. Wayne Streilein, J. Richard Taylor, Iwao Kurimoto, Tadamichi Shimizu, Cynthia Tie, and Cindy Golomb, University of Miami School of Medicine, Miami, Florida.

Acute, low dose ultraviolet B (UVB) radiation followed by epicutaneous application of dinitrochlorobenzene (DNCB - 2000 µg) identifies a subpopulation of normal human beings who fail to develop contact hypersensitivity (40% of subjects, N = 56). These individuals are termed UVB-Susceptible (UVB-S), whereas those that develop contact hypersensitivity when hapten is painted on UVB-exposed skin are termed UVB-Resistant (UVB-R). Among UVB-S subjects, a subset (9%) displays UVB-dependent, hapten-specific tolerance. In addition, approximately 50% of UVB-R volunteers acquire a sustained, immunosuppressive microenvironment at the UVB-exposed site which prevents the local expression of contact hypersensitivity. A polymorphism has been detected in the 5' untranslated region of the human *Tnfα* locus in which nucleotides *T* or *C* occupy position 1028 upstream from the gene's strart site. Since a *C* at this position correlates with the UVB-S phenotype, and since individuals who are UVB-S when tested with DNCB are usually UVB-S when tested with the non-cross reacting hapten diphencyprone (>80% concordance rate), the traits of UVB-S and UVB-R appear to be genetically determined. When patients with biopsy-proven basal or squamous cell skin cancer or with malignant melanoma were studied, a very high proportion of the former (92%, N = 41), and all of the latter (N = 11) proved to be UVB-S. When tested for hapten-specific tolerance, 50% of these cancer patients were also unable to acquire DNCB-specific contact hypersensitivity. Similarly, when patients with recurrent herpes labialis were tested, a high frequency of subjects (67%, N = 23) proved to be UVB-S. Based on these data, we propose that the effects of UVB on cutaneous immunity in man are genetically determined, that molecular polymorphisms at the *Tnfα* locus predict the dimorphic phenotypes of UVB-S and UVB-R, and that the UVB-S trait is an important risk factor for sunlight-induced skin cancers, and for sun-related recurrent herpes labialis.

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POSTTRANSCRIPTIONAL REGULATION OF INTERLEUKIN (IL)-6 GENE EXPRESSION IN HUMAN KERATINOCYTES (KC) BY ULTRAVIOLET (UV) B RADIATION. Jean Krutmann, Sven de Voss*, Marion Brach*, Uwe Trefzer, Anne Budnik, and Friedhelm Herrmann*, Department of Dermatology and *Medicine, University of Freiburg, Germany.

UVB-induced local and systemic inflammatory reactions are thought to be mediated at least in part by UVB-induced KC-derived IL-6. Previously, UVB-induced KC IL-6 production was found to be associated with an enhanced expression of steady state levels of IL-6 mRNA. In order to determine whether UVB light would increase IL-6 mRNA expression via transcriptional and/or posttranscriptional mechanisms, in the present study, 4th passage, normal human keratinocytes (HNK) were exposed in vitro to UVB light (0-100 J/m^2) from FS 20 sunlamps. As expected, UVB exposure of HNK significantly enhanced low, constitutive expression of IL-6 mRNA and secretion of IL-6 protein in a time- and dose-dependent manner. Using an in vitro nuclear transcription assay, transcription rates of IL-6 genes in nuclei isolated from UVB-irradiated HNK were found to be essentially identical to those from unirradiated cells, indicating that UVB light did not initiate new transcription, but rather might act via a posttranscriptional mechanism involving mRNA stability. Irradiated and unirradiated HNK were therefore treated with actinomycin D, and mRNA half-life times calculated from Northern blotting experiments. As compared to unirradiated cells, IL-6 mRNA stability was significantly increased (3-fold) in UVB-irradiated cells. This is the first report indicating that UVB light upregulates IL-6 mRNA levels in HNK at a posttranscriptional level by increasing stability of mRNA.

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UVB LIGHT INDUCES NF-KAPPA-B (NFkB) ACTIVATION BY CYTOSOLIC NFkB-IkB COMPLEX DISSOCIATION IN DNA FREE CYTOSOLIC PROTEIN EXTRACTS OF HUMAN EPIDERMAL CELLS. Manuel Simon, Yoshinori Aragane, Agatha Schwarz, Thomas Luger, Thomas Schwarz, Ludwig Boltzmann Institute of Cellbiology and Immunobiology, Dept. Dermatology, University of Muenster, DW-4400 Muenster, Germany

NFkB is a DNA binding protein, known to be activated by a diverse set of different stimuli. A recognition sequence for this transcriptional activator was found in a variety of promoters of stress response genes including the IL6 gene. We have recently shown this protein complex to be involved in signaling the low dose UVB stimulus in A431 cells (epidermoid carcinoma cell line). A fusion construct of an IL6 promoter fragment, containing a NFkB recognition sequence (-110 to +13) with the CAT gene, transfected to the epidermal carcinoma cell line KB was shown to mediate induction of CAT expression after UVB irradiation. NFkB was previously reported to bind an inhibitory protein, IkB. The NFkB-IkB complex exhibits no DNA binding activity and is localized in the cytoplasm. Once activated, the complex dissociates, binding active NFkB migrates into the nucleus and activates gene transcription. NFkB binding activity is demonstrated to be present in the cyotosol obtained from low dose UVB treated cells compared to the untreated control. To investigate whether this effect is mediated by signals like DNA damage or IL-1, known to stimulate NFkB activity, cytosolic protein extracts from unirradiated A431 cells were exposed to UVB light. NFkB activity was increased upon UVB irradiation in a fast, dose dependent manner. The extracts contained no PCR-detectable genomic DNA contamination. These observations suggest a cytosolic UVB-target. One mechanism of activating NFkB was reported to involve oxygen radicals. PDTC, a radical scavenging compound, was shown to be toxic to keratinocyte cell lines in µM amounts. Sublethal concentrations abolished the UVB dependent NFkB activation, but activated basal binding activity in untreated cells, suggesting oxygen radicals to be necessary second messengers.

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UVB IRRADIATION INDUCES CYTOSOLIC PHOSPHOLIPASE A_2 SYNTHESIS IN HUMAN KERATINOCYTES. Alice P Pentland*¥, Jaime Masferrar† and Alane Gresham*, Division of Dermatology, Department of Medicine, Washington University School of Medicine; †Monsanto Corporation, St. Louis, MO. U.S.A.

Exposure to UVB radiation induces acute inflammation characterized by erythema and edema. At early time points after irradiation, erythema is mediated primarily by PGE_2. This increased PGE_2 synthesis is the result of increased phospholipase activity. Several cytosolic phospholipases ($cPLA_2$) have recently been described which are likely to regulate arachidonate release related to signal transduction. To determine if keratinocytes synthesize $cPLA_2$, and the effect of UVB on enzyme synthesis and distribution, we have raised rabbit polyclonal antibodies against 15-mer sequences of the N-terminus and the Ca^{++}-phospholipid binding site of human monocyte $cPLA_2$. Human keratinocytes cultures were prepared and studied 2 days post-confluence. Both antisera blocked the hydrolysis of arachidonate from 1-palmitoyl-2-[arachidonyl-1-^{14}C] phosphatidylcholine by keratinocyte homogenates, demonstrating the antisera are directed toward a phospholipase. Keratinocytes were metabolically labeled and harvested at intervals after exposure to 30 mJ/cm^2 UVB. Both antibodies immunoprecipitated an identical single band of approximately 105 kDa. Synthesis of $cPLA_2$ increased 6 hr. after irradiation, was maximal by 9 hr. and returned to baseline 24 hr. post-UV. In-vivo localization of $cPLA_2$ was determined by standard immunohistochemical techniques with both $cPLA_2$ antisera. Analogous staining patterns were observed for both antibodies: $cPLA_2$ is localized in the stratum malpighii in unirradiated skin, but is dramatically increased in the entire superbasal region following irradiation. A cDNA prepared from reverse transcribed keratinocyte mRNA was sequenced and found to be homologous to the monocyte enzyme over a 564 bp region including the Ca^{++} binding and active sites. Our results suggest that the cytosolic form of PLA_2 may play a vital role in epidermal response to acute UVB injury.

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DIFFERENTIAL EFFECTS OF SUNSCREENS AND A DNA EXCISION REPAIR ENZYME ON ULTRAVIOLET RADIATION-INDUCED SKIN EDEMA, SUNBURN CELL FORMATION, AND DAMAGE TO CUTANEOUS IMMUNE CELLS. Peter Wolf, Patricia Cox, Daniel B. Yarosh*, and Margaret L. Kripke, Dept. of Immunology, U.T.M.D. Anderson Cancer Center, Houston, TX, and *Applied Genetics Inc., Freeport, NY

Exposure of skin to ultraviolet radiation (UVR) leads to various alterations, which include inflammation, histopathologic changes, and damage to cutaneous immune cells. Although the molecular mechanisms of UV-induced alterations are not completely understood, DNA damage is thought to play an important role. In the present study, we investigated the protective effects of sunscreens containing 8% octyl-N-dimethyl-p-aminobenzoate, 7.5% 2-ethylhexyl-p-methoxycinnamate, or 6% benzophenone-3, and a liposome suspension containing T4 endonuclease V (T4N5), a cylobutane pyrimidine dimer (CPD)-specific DNA repair enzyme, on UV-induced skin edema, sunburn cell (SBC) formation, and damage to $ATPase^+$ and Ia^+ Langerhans cells (LC) and $Thy\text{-}1^+$ dentritic epidermal cells (dEC). Exposure of C3H mice to a single dose of 500 mJ/cm^2 UVB radiation from FS40 sunlamps resulted in significant skin edema, SBC formation, and morphologic alterations and 48-67% decrease in the number of LC and dEC in the epidermis. Application of the sunscreens before UV irradiation gave almost complete protection against UV-induced edema, SBC formation, and damage to LC and dEC; the sunscreen vehicle alone was ineffective. In contrast, the topical application of T4N5 liposomes after UV irradiation only moderately affected UV-induced skin edema, but it reduced the frequency of SBCs by approximately 50%, and almost completely prevented morphologic alterations and decrease of LC and dEC. The application of heat-inactivated liposomes had no effect on these UV-induced alterations. These findings indicate that although both sunscreens and T4N5 liposomes reduce DNA damage, they have overlapping, but distinct effects on various cutaneous responses to UVR. Sunscreens and T4N5 liposomes equally protect against damage to cutaneous immune cells, whereas sunscreens better protect against inflammation and SBC formation. Moreover, the results demonstrate that the molecular mechanisms of SBC formation and damage to cutaneous immune cells involve CPD. Besides the application of sunscreens before UV exposure the use of liposomes to deliver lesion-specific repair enzymes to the skin *in situ* afterwards may be a useful way to reduce the negative effects of UVR even after a sunburn reaction has been initiated.

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WAVELENGTH-SPECIFIC INDUCTION OF IMMEDIATE EARLY GENES IN KERATINOCYTES BY ULTRAVIOLET RADIATION. K Ariizumi, PR Bergstresser, A Takashima UT Southwestern Med Center, Dallas, TX, USA.

Exposure of skin to UVB and to UVA radiation produces clinically distinct changes; UV radiation in these ranges also produces distinct changes in cellular function. Immediate early genes are a specific set of genes that are activated most rapidly in response to exogenous stimuli. Products of these genes serve as nuclear mediators by binding to promoter regions (e.g., AP-1 site), thereby regulating the transcription of other functional genes. We thus hypothesized that UVB and UVA induce the expression of different sets of immediate early genes in keratinocytes (KC). A human KC line, A431, was exposed to either UVB (FS-20, 25-400J/m^2) or UVA (black light, 2.5-20 kJ/m^2) and then examined by Northern blotting. Rapid upregulation of fra-1 (10-fold after 60 min) was observed after UVA radiation, but not after UVB radiation. UVA exposure also upregulated c-myc mRNA (4-fold), while UVB downregulated this expression. Conversely, c-jun was markedly upregulated (10-fold) only with UVB exposure. No changes were detected in mRNA levels for jun-B, jun-D or fos-B in response to either source of radiation. UV dose-response studies indicated that the optimal upregulation of each gene required similar doses; 100 J/m^2 for UVB and 10 kJ/m^2 for UVA. Kinetic experiments demonstrated a relatively rapid onset, with significant changes detectable as early as 15 min after UV exposure and increasing thereafter. UVB-induced c-jun upregulation or c-myc downregulation was no longer detectable in cells pretreated with superoxide dismutase, indicating that reactive oxygen species are involved in UVB signaling. Treatment of cells with DNA alkylating reagents failed to modulate c-jun, c-myc or fra-1 expression, suggesting that DNA damage per se does not activate UVB or UVA signaling pathways. These results document that UVB and UVA radiation induce the expression of distinct sets of immediate early genes in KC, supporting the hypothesis that disparate signaling pathways are activated by different wavelengths within the UV spectrum.

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MUTATION AND OVEREXPRESSION OF p53 GENE IN HUMAN SKIN CANCER OF SUN-EXPOSED AREA. Chikako Nishigori, Hiraku Takebe*, Yasuhiro Matsumura and Sadao Imamura, Departments of Dermatology and *Experimental Radiology, Kyoto University Faculty of Medicine, Kyoto, Japan

Possible involvement of p53 gene mutation and overexpression in human skin cancer in the sun-exposed area, presumably due to UV-specific alterations in DNA, was investigated. DNA extracted from 23 skin tumors in 13 xeroderma pigmentosum (XP, 6 Tunisian and 7 Japanese) patients and 11 skin tumors in 9 non-XP Japanese subjects were analyzed. Two methods, immunohistochemical staining with anti-p53 antibodies (CM-1, PAb 1801, and PAb421) and the single strand conformation polymorphism (SSCP) analysis for exon 4 through 8, were used. Positive stainings for anti-p53 antibodies were detected in 13 tumors. Among the 23 tumors from XP patients, 11 tumors were detected to have abnormal shifts in the SSCP analysis indicating that there are mutations in the p53 gene. Among 11 tumors in the non-XP subjects, 6 were found to have mutations. There was a good correlation between anti-p53 antibody positive tumors and tumors with abnormal shift in SSCP. By nucleotide sequencing of the SSCP-shifted DNA, transitions were more often (approximately 60 %) found than transversions, and most of the mutated sites corresponded to the dipyrimidines. These results suggest that abnormal function in p53 gene via UV specific mutation should play a role in the UV carcinogenesis.

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TOPICAL δ-AMINOLEVULINIC ACID FOR PHOTODYNAMIC THERAPY OF CUTANEOUS CARCINOMAS AND CUTANEOUS T CELL LYMPHOMA. SD Shanler, W Wan, JE Whitaker, TS Mang, C Jones, BD Wilson, HL Stoll, S Pincus and AR Oseroff. Roswell Park Cancer Inst, and SUNY at Buffalo, Buffalo, NY

A new approach to photodynamic therapy (PDT) of superficial malignancies employs topical δ-aminolevulinic acid (ALA), which is biosynthetically converted to the photosensitizer protoporphyrin IX (PP). Therapeutic selectivity is due to enhanced ALA penetration across abnormal stratum corneum and to preferential accumulation of PP in epidermally derived cells, compared to fibroblasts and endothelial cells. We investigated PDT with topical ALA in superficial basal cell carcinomas (S-BCC), and patch/plaque stage cutaneous T-cell lymphoma (CTCL). 2%-40% ALA was applied under occlusion for 4-5 hours; in some cases tape-stripping or iontophoresis was used to enhance uptake. PP was measured by *in situ* fluorescence, and by intensified video fluorescence microscopy on frozen sections. Three-six fold more PP accumulated in lesions than in surrounding skin. In 30 patients with S-BCC followed for up to 90 weeks, ALA concentrations of 10%-40% and 75-200 J/cm^2 630 nm light gave 100% complete initial responses with excellent cosmetic results and minimal scarring. Despite PP photobleaching by therapeutic light doses, significant recovery of PP fluorescence was observed in the lesions. In lesions with normal stratum corneum, ALA uptake and PDT selectivity and efficacy were reduced, but tape stripping and iontophoresis were helpful. Both direct phototoxicity and significant host inflammatory reactions contributed to the therapeutic response. In CTCL, PP preferentially accumulated within lymphocytic infiltrates, and early therapeutic responses suggest prolonged remissions from single treatments. Thus, topical ALA is promising for PDT of superficial cutaneous malignancies.

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DIFFERENTIAL DAMAGES OF UV EXPOSURES TO THREE DIMENSIONAL NETWORKS OF ELASTIC FIBERS DURING MATURATION OF RAT SKIN. Yoshinori Takema, Kazue Tsukahara, Shuhei Imayama and Genji Imokawa Kao Biological Science Laboratories, Tochigi, and Department of Dermatology, Kyusyu University, Kyusyu, Japan.

We previously demonstrated that the loss of three-dimensional linearity of elastic fiber is predominantly associated with the reduction of skin elasticity and wrinkle formation during photoaging process. In order to elucidate differential effects of UV exposures on three dimensional networks of elastic fibers during maturation of rat skin, SD-RAT hind limbs were irradiated with sub-erythemal dose of UV lights(UVB: SE-20 lamp, 130 mJ/day; or UVA: BLB lamp, 27J/day) in 3 different time courses of exposures : 3-9 weeks old, 9-15 weeks old and 3-15 weeks old .Three dimensional arrangement especially referring to linearity of elastic fiber was quantified by image analysis in scanning electron microscopy observed after a combination of intravascular resin injection and selective digestion technique using formic acid. Six weeks of UV exposures(UVB, 3 times per weeks; UVA, 5 times per week) from 3 weeks old caused SD-RAT hind limbs skin to significantly increased tortuous and irregularity of elastic fiber under SEM observations compared with the same exposure period beginning from 9 weeks old, the magnitude of which was larger by UVB exposure than that by UVA exposure. After termination of UV exposures at 9 weeks old, there was no restoration in deranged three dimensional structure of elastic fibers by 15 weeks old. These findings indicate that UV exposures during the early maturation stage is most susceptible to the disruption of three-dimensional linearity of elastic fiber.

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SUPERANTIGENS DERIVED FROM SKIN COLONIZING STAPHYLOCOCCI PROFOUNDLY INFLUENCE IMMUNOLOGICAL REACTIONS OF THE SKIN

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Superantigens like Staphylococcal Enterotoxin B (SEB), which is produced among others by skin colonizing staphylococci, are potent modulators of the immune system, as they can interact with all T cells expressing certain Vß elements as part of their antigen receptor and antigen presenting cells expressing MHC class II molecules. Therefore we studied the effect of SEB on normal skin and on other immunological reactions takeing place in the skin of BALB/c mice.

A single application of SEB (intradermal injection of 50 ng SEB) was able to elicit a strong inflammatory response in the skin: Increased endocytotic activation of Langerhans cells and mast cell degranulation (electron microscopy), vasodilation, induction (VCAM-1)/up-regulation (ICAM-1) of adhesion molecules (immuno histology) after 3 to 6 hours and a strong infiltration by granulocytes followed by mononuclear cells after 12 to 24 hours. The total cell number of the regional lymph nodes was increased up to 10-fold within 24 to 48 hours, with an additional relative over-expression of Vß8$^+$ T-cells (flow cytometry). Multiple injections (8-times 50 ng SEB i.d. over 14 days) lead to a 30% decrease of Vß8$^+$ T-cells among regional draining lymph node T-cells and an even stronger functional inhibition of these cells (80% decreased proliferative responses to SEB or cross-linked Vß8-antibodies in vitro).
If such multiple injections with SEB were given during the induction of immediate hypersensitivity to ovalbumin (OVA)(by skin painting of the shaved and alcohol abraded skin with OVA 50 mg/ml in PBS 8-times over two weeks), which - as we had demonstrated earlier - was much dependent on help provided by Vß8$^+$ T-cells, the induction of OVA-specific IgE in the sera (ELISA), skin test responses to OVA (i.d. injections of OVA) and bronchial hyperresponsiveness after challenge with OVA (electrical field stimulation of tracheal segments) was blocked. In contrast, if a single SEB injection was given one day before skin painting with 2,4-dinotrofluorbenzene (DNFB) (0,2% DNFB in oil/acetone painted once on the shaved skin), induction of delayed hypersensitivity to DNFB was enhanced (measurement of ear swelling 24 hours after challenge with DNFB 0,2% in oil/acetone).

These data indicate that superantigens from skin colonizing bacteria profoundly influence (local) T cell repertoire and function favoring T_{H1}-type over T_{H2}-type responses. The latter is also backed by the observations that SEB treatment increases IFN-γ but not IL-4 production of regional lymph node cells in vitro (ELISA-kits) and that the OVA-specific immunoglobuline response after OVA exposure was shifted by SEB treatment from IgE and IgG_1 (T_{H2}-dependent) to IgG_{2A} and others (T_{H1}-dependent).

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SUPPRESSIVE EFFECT OF STAPHYLOCOCCAL ENTEROTOXIN B ON MURINE CONTACT HYPERSENSITIVITY. Kayoko Abe, Masaru Natsuaki, Yukio Kitano, Department of Dermatology, Hyogo College of Medicine, Hyogo, Japan

Staphylococcal enterotoxins (SE) are well known superantigens (SA), which interact with particular Vß regions of T cell recepter and major histocompatibility complex class II molecules, and activate large number of T cells. Therefore, immune responses may be modulated by SA. In this study, we investigated the effect of SEB on contact hypersensitivity reaction (CHR) in mice. As the methods, BALB/c mice were sensitized with dinitrofluorobenzene (DNFB) on their shaved back skin on day 0 and challenged with DNFB on the left ear on day 5. Ear swelling was measured at 24, 48, 72 hours after the challenge. 50μg of SEB was intravenously injected to each mouse at various times from day -7 to day 5. Regional lymph node (LN) cells from these mice were cultured in vitro with the same hapten or SEB and the proliferative response was quantified by measuring the incorporation of 3H-thymidine. As the results, CHR was suppressed by SEB-injection on day 0 but not on day -7, -4, 3, 5. In contrast, CHR in the mice pretreated with SEB on day -7 was not suppressed even if SEB was injected on day 0. Spleen cells derived from SEB-injected mice did not transfer the suppression to naive recipient mice. In in vitro study, the proliferative response of LN cells to the specific hapten was suppressed in the mice treated with SEB on day 0, while it was not suppressed in the SEB-pretreated mice. In vitro stimulation with SEB induced significant proliferation in naive LN cells but not in the SEB-pretreated cells. This indicates that SEB-sensitive cells once stimulated by SEB do not respond to SEB any more. These results suggest that the injection of SEB on day 0 could induce a suppression of CHR, in which not suppressor cells but SEB-responding cells may play an important role.

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FLARE-UP REACTION INDUCED BY STAPHYLOCOCCAL ENTEROTOXIN B ON MURINE CONTACT HYPERSENSITIVITY. Masaru Natsuaki, Kayoko Abe, Noriko Yamashita, Yukio Kitano, Department of Dermatology, Hyogo College of Medicine, Hyogo, Japan

Staphylococcal enterotoxins (SE) have been defined as superantigens (SA). These interact with particular Vβ regions of T cell recepter and major histocompatibility complex classII molecules, and activate large number of T cells. In this study, we investigated whether SEB activates the immunological memory of contact hypersensitivity (CH) in mice. As the methods, BALB/c mice were sensitized with dinitrofluorobenzene (DNFB) or oxazolone (Ox) on their shaved back skin on day 0 and challenged with the same hapten on the left ear on day 5. CH reaction (ear swelling) was recognized on day 6. Intravenous injection of a specific hapten on day 33 induced a flare-up reaction only on the left ear. This indicates that a hapten specific immunological memory was persisted in the CH-responded site. In contrast, intravenous administration of SEB on day 33 induced marked left ear swelling with a peak response at 24 hours after the injection in both DNFB-sensitized and Ox-sensitized mice. These results suggest that SEB might stimulate memory cells of CH reaction without hapten specificity and thus, SA may explain the mechanism of flare-up reactions associated with bacterial infection.

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IMMUNOTHERAPY OF CUTANEOUS TUMORS WITH THE BACTERIAL SUPERANTIGEN STAPHYLOCOCCAL ENTEROTOXIN B. CA Elmets, WS Mirando, SI Zaidi, K Tubesing, H Mukhtar. Case Western Reserve University, Cleveland, OH USA.

T lymphocytes play a critical role in the host response to cutaneous squamous cell carcinomas and strategies that augment the T cell response to tumors have been shown to cause tumor regression. Because the bacterial superantigen staphylococcal enterotoxin B (SEB) is an exceptionally potent T cell activating stimulus, its efficacy as an immunotherapeutic agent for cutaneous tumors was investigated. In *in vitro* studies, a dose dependent reduction in tumor cell growth was observed when the SCC13 squamous cell carcinoma cell line was incubated with purified T lymphocytes and SEB. Five ng/ml SEB inhibited tumor cell proliferation by over 85% (166,000 ± 19,000 cpm vs 19,000 ± 3,000 cpm). Both SEB and T cells were necessary for inhibition of tumor cell growth since no reduction in SCC 13 cell growth was observed when either was omitted from the cultures. X-irradiation of T cells with 2500 R failed to abrogate their tumor growth inhibitory effect; however, no reduction in tumor growth was observed when formaldehyde fixed T cells were used in the cultures, indicating that viable, radioresistant T cells were necessary for this effect. To investigate the *in vivo* significance of this phenomenon, BALB/c mice bearing intracutaneous transplanted PAM 212 squamous cell carcinomas were given an intratumor injections of 100 micrograms SEB on one to three occasions and the size of the tumors were evaluated over subsequent days. Treatment in such a manner resulted in 75-100% tumor regression. These studies demonstrate that bacterial superantigens have the potential to act as immunotherapeutic agents for cutaneous tumors and that the effect is likely to be mediated through its T cell activating properties.

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PGE_2 INHIBITION & INTERFERON-GAMMA RESTORATION OF TYPE 1 T CELL GROWTH RATE IN ATOPIC DERMATITIS. Sai C. Chan, Shi-Hua Li, William R. Henderson, Jr., and Jon M. Hanifin, Dept. of Dermatology, Oregon Health Sciences University, Portland, OR, and Dept. of Medicine, University of Washington, Seattle, WA.

The phytohemagglutinin (PHA)-stimulated proliferative rate is lower in peripheral blood mononuclear cells (PBMC) from patients with atopic dermatitis (AD). We considered that the elevated PGE_2 and IL-4 production by AD leukocytes might inhibit proliferation of the predominant Type 1 CD_4^+ T cell subset (Th1), and that interferon-gamma (IFN-γ) could reverse the inhibition.

PBMC were isolated on Hypaque Ficoll gradients, then suspended in medium ± PHA, IFN-γ or IL-4. Proliferative index (PI) was calculated by ^{3}H-TdR uptake (CPM) in PHA-stimulated/unstimulated MNL. Twenty-four-hour culture supernatants were also assayed for IL-4 (by ELISA) and for PGE_2 and IFN-γ (by RIA).

When compared to normals, PGE_2 levels were increased ($p=0.014$) in AD cultures which had a reduced PI ($p<0.05$) correlating with low IFN-γ ($r=0.99$) and inversely with IL-4 ($r=0.65$). Addition of IFN-γ (100 u/ml) caused a 222% increase of PI in AD cell cultures ($p<0.05$), while having a negligible effect on normal cells. Addition of IL-4 (100 u/ml) reduced PI in AD but not normal cultures.

Increased PGE_2 may relate to elevated IL-4 production by Th2 in AD, inhibiting Th1 growth and causing low level of IFN-γ production. Exogenous IFN-γ restores a normal Type 1 T cell growth rate, possibly by reducing IL-4 synthesis. This may represent a mechanism by which IFN-γ therapy can normalize the Th1:Th2 imbalance and decrease inflammation in atopic dermatitis.

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IN VITRO MODULATION OF EPIDERMAL INFLAMMATORY CYTOKINES (IL-1α, IL-6, TNFα) BY MINOCYCLINE. Brigitte Dréno, Philippe Célérier, Pierre Litoux, Department of Dermatology, Nantes, Hôtel-Dieu, France.

Systemic administration of minocycline has been shown to be effective in the treatment of acne vulgaris specifically on inflammatory lesions. One of the direct anti-inflammatory effects is a suppressive effect on the chemotaxis of human leukocytes but this action could also be related to an inhibition of inflammatory cytokines produced by the keratinocytes. So we investigated the in vitro modulation of inflammatory cytokines (IL-1α, IL-6, TNFα) by minocycline.

Using a reconstituted skin (RS) model, we studied mRNA and protein epidermal production of IL-1α, IL-6 and TNFα. RS obtained from inflammatory skin (eczema) and control skin were cultivated in the presence or absence of minocycline. (1μg/ml) during 10 days. Then, on frozen sections, an immunohistochemical study using monoclonal antibodies labelled with an immuno peroxydase technique (anti IL-1α, IL-6, TNFα) and an in situ hybridization using biotylinated cold probes were respectively performed for the analysis of protein and mRNA cytokines production.

This study shows that minocycline decreases TNFα epidermal production (mRNA and protein levels) both in inflammatory and control skins, do not modify IL-6 production and paradoxically increases IL-1α production (mRNA and protein level) both in inflammatory and control skins.

So theses results indicate that a part of anti-inflammatory effect of minocycline could be related to a decrease of TNFα epidermal production. Interestingly, the inducing effect of minocycline in vitro on IL-1 production by keratinocytes is similar to these previously reported with monocytes (Ingham 1990) and on comedonal IL-1 level in acne patients treated with tetracyclines (Eady 1991).

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NEUROKININ MODULATION OF NORMAL HUMAN KERATINOCYTE CYTOKINE PRODUCTION. K.J. Sung, A.H. Kaynard, J. Brown, C.A. Armstrong, J.C. Ansel. Dermatology Service, Veterans Affairs Medical Center, and Dermatology Department, Oregon Health Sciences University, Portland, OR

Recent evidence indicates that neurokinins released from cutaneous C-fibers can function as inflammatory mediators by activating cells of the immune system such as lymphocytes, monocytes, and mast cells. We have previously shown that substance P (SP) specifically induces mast cells to produce the cytokine TNFα. In the present study we tested the hypothesis that SP may also mediate inflammatory changes in the skin by specifically activating human epidermal keratinocytes to secrete potent bioactive cytokines. We found that the addition of SP to cultured normal human foreskin keratinocytes results in a rapid bimodal increase in intracellular Ca^{++} followed by a dose dependent increase (1, 10, 100, to 1000 nM SP) in keratinocyte IL-1α, IL-6, and TGFα mRNA levels after one hour. SP has no effect on keratinocyte TNFα or IL-8 production. Kinetic studies demonstrate that keratinocyte IL-1α, IL-6, and TGFα mRNA are maximally increased 3 hours after SP stimulation and return to constitutive levels by 6 hours post induction. SP structure-function studies indicate that SP_{1-9} but not SP_{4-11} or SP_{7-11} peptide fragments induce keratinocyte cytokine production. *In vivo* studies with capsacian treated human skin demonstrate by immunohistochemistry that SP is rapidly released into both the dermis and epidermis followed by a marked increase in epidermal IL-1 production 12 hours after capsacian application. These studies further support the role of the neurologic system in cutaneous inflammation.

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UPREGULATION OF THE NEUROPEPTIDES, SUBSTANCE P (SP) AND CALCITONIN GENE-RELATED PEPTIDE (CGRP), IN PRIMARY SENSORY NEURONS FOLLOWING CUTANEOUS INFLAMMATION. T.A. Leslie[1,2], P.C. Emson[3], C.J. Woolf[2] and P.M. Dowd[1]. Dept. of Dermatology[1] and Dept. of Anatomy[2], University College London School of Medicine[2], UK.MRC group, Babraham, Cambridge UK[3].

We have examined the effect of peripheral inflammation on the expression in primary sensory neurons, of the neuropeptides, substance P (SP) and calcitonin gene-related peptide (CGRP). These neuropeptides are contained in primary afferent neurones and have been implicated as mediators in the neurogenic mechanisms of cutaneous inflammation and the axon-reflex flare. They produce plasma extravasation with vasodilatation. CGRP is also thought to modulate antigen presentation to Langerhans cells. The expression of SP and CGRP mRNA in adult rat dorsal root ganglia (DRG) was examined using a non-radioisotopic *in situ*-hybridisation technique with alkaline phosphatase-linked oligonucleotide probes. Ipsilateral and contralateral lumbar DRGs were examined at 6, 24, 48 and 120 hours after turpentine and complete Freund's adjuvant (CFA) injections into the left hind-paw to produce cutaneous inflammation. Both models produced hyperalgesia and local swelling. Hindpaw inflammation caused an increase of SP and CGRP mRNA in the ipsilateral L4 DRGs within 6 hours of turpentine and CFA injection, this remained above control levels at 120 hours. In the contralateral L4 DRGs there was an increase in SP mRNA after 48 hours. The SP mRNA levels in both ipsilateral and contralateral L3 DRGs remained at control levels . This upregulation of SP and CGRP may be consequent to the production of neurally active inflammatory cytokines or neurotrophins in the periphery and might constitute a possible feedback mechanism, whereby inflammation upregulates the factors responsible for neurogenic inflammation and, since neuropeptides are mitogenic (Hanley MR. *Nature* 1985; **315**:14-15), for keratinocyte hyperproliferation. These neuropeptide responses may mediate functional and even subsequent structural alterations in peripheral cutaneous innervation and be of great importance in modulating the inflammatory response.

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MODULATION OF INTERLEUKIN-8 SECRETION AND EICOSANOID METABOLISM IN CULTURED SYNOVIAL FLUID MONONUCLEAR CELLS FROM PSORIATIC ARTHRITIS BY INTERLEUKIN-4 AND INTERLEUKIN-10. Kragballe K, Deleuran B, Iversen L, Kristensen M, Thestrup-Pedersen K, Stengaard K. Department of Dermatology and Rheumatology, University Hospital of Aarhus, Aarhus, Denmark.

There is increasing evidence that the proinflammatory cytokines and eicosanoids play a role in the pathogenesis of psoriatic arthritis. The purpose of this study was to compare the ability of interleukin-4 (IL-4) and interleukin 10 (IL-10) to regulate the spontaneous secretion of interleukin-8 (IL-8) in cultured synovial fluid mononuclear cells from patients with psoriatic arthritis. Furthermore, to assess whether IL-4 and IL-10 could alter the production of eicosanoids by synovial cells. Synovial fluid mononuclear cells from psoriatic arthritic joints were cultured in the absence or presence of human recombinant IL-4 or human recombinant and human biological IL-10. IL-8 secretion was measured by ELISA in the culture supernatants, and eicosanoids by reverse phase-HPLC. After incubation for 72 hours the spontaneous IL-8 secretion was suppressed by IL-4 (10 ng/ml) to 25%, by recombinant IL-10 (10 ng/ml) to 65% and by biological IL-10 (10 U/ml) to 40%. The formation of eicosanoids was determined in synovial fluid cells stimulated with arachidonic acid (25 μM) and A23187 (5 μM). IL-4, but not IL-10, stimulated the formation of 15-hydroxy-eicosatetraenoic acid (15-HETE) (23 ng/10^6 cells), which was not detectable in untreated cells. These results show that IL-10, but in particular IL-4, can suppress the secretion of the proinflammatory IL-8, and that IL-4 selectively can induce the formation of the anti-inflammatory 15-HETE in cultured cells synovial fluid mononuclear cells with psoriatic arthritis. It is possible that IL-4 and IL-10 treatment could be used to control the inflammation in psoriatic arthritis.

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ULTRAVIOLET B LIGHT IMPAIRS CONTACT HYPERSENSITIVITY AND INDUCES TOLERANCE BY DIFFERENT IMMUNE MECHANISMS. Tadamichi Shimizu and J. Wayne Streilein, University of Miami School of Medicine, Miami, FLorida, and Hokkaido University School of Medicine, Sapporo, Japan.

Ultraviolet B (UVB) radiation impairs contact hypersensitivity (CH) induction in certain strains of mice (UVB-susceptible - UVB-S) by converting trans- to cis-urocanic acid (UCA) which in turn causes TNFα to accumulate in the skin. In this microenvironment, hapten-bearing antigen presenting cells are immobilized and can *not* carry the immunogenic signal to the draining lymph node. CH can be restored in UVB-S mice if they are treated systemically with a neutralizing anti TNFα antibody at the time hapten is painted on UVB-exposed skin. Similarly, CH is restored in mice that receive an epicutaneous application of hapten to skin injected at the application site with TNFα (50 ng) or cis-UCA (200 μg), indicating that CH is disrupted after UVB, cis-UCA and TNFα via the actions of TNFα itself. In UVB-S mice, UVB radiation induces hapten-specific tolerance, an outcome that is similarly achieved when hapten is painted on skin that has received intracutaneous injections of sub-inflammatory doses of cis-UCA or TNFα. Moreover, all recipients (UVB-S and UVB-R) treated with UVB, cis-UCA, or TNFα develop hapten-specific T suppressor cells. However, anti TNFα antibodies *fail* to reverse tolerance that follows hapten application to skin treated with UVB or cis-UCA, although these antibodies do prevent TNFα-induced tolerance. To demonstrate that impaired CH and tolerance develop by different mechanisms, hapten was painted simultaneously on UVB-exposed and unexposed skin of UVB-S mice. On the one hand, these mice developed vigorous CH, but upon re-immunization with the same hapten, they displayed tolerance. We conclude that UVB impairs CH induction by a TNFα-dependent pathway that is strictly local and passive, whereas UVB promotes tolerance by a TNFα-independent mechanism that is active, systemic, and initiated by blood-borne cells recruited to the UVB-damaged dermis.

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INFLAMMATORY STIMULI ARE POTENT INDUCERS OF HEAT SHOCK RESPONSE IN HUMAN KERATINOCYTES. Axel Beetz, Manfred Köller, Günter Michel, Ralf-Uwe Peter, Lajos Kemény, Wolfgang König, Thomas Ruzicka. Dept. of Dermatology, Univ. of Munich, Dept. of Immunology, Univ. of Bochum, Germany, Univ. of Szeged, Hungary.

Heat shock response has first been described in cells or organisms that were exposed to elevated temperatures. This response turned out to be universal and comprises a small number of highly conserved proteins (hsps). These can also be induced by challenge with ethanol, sulfhydryl reagents, heavy metal ions and a variety of viruses.

In human keratinocytes (KC) induction of hsps has been shown by heat and UV treatment, but the physiological stimuli for hsp production in skin are unknown. We show here for the first time that mediators and cytokines normally associated with inflammatory processes as well as ionizing radiation are potent inducers of hsps in human KC.

The transformed human epidermal cell line SCL II was exposed to the inflammatory mediators IL-8 (20nM), TNFα (0.3nM), 12-HETE (25nM), as well as to gamma-radiation (6Gy). After 30, 60, 120 and 240 min. total RNA and protein were extracted and analysed by Northern and Western blotting for the presence of hsp 72.

All treatments induced synthesis of hsps, however with a different time course. TNFα and 12-HETE led to an early response, comparable to heat induction, with maximum hsp 72 expression at 1hr. In contrast, a delayed response was seen with IL-8 and gamma-radiation with highest hsp 72 levels at 4hrs.

These experiments suggest that besides physical stimuli such as heat and ionizing radiation, inflammatory mediators and cytokines are potent inducers of heat shock response in human KC.

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IDENTIFICATION AND SUBCELLULAR LOCALIZATION OF LEUKOTRIENE A_4 HYDROLASE ACTIVITY IN HUMAN EPIDERMIS. Lars Iversen, Vincent A. Ziboh, Olof Rådmark, Anders Wetterholm and Knud Kragballe. Department of Dermatology, Marselisborg Hospital, University of Aarhus, Aarhus, Denmark (LI, KK). Department of Dermatology, University of California, Davis, California, USA (VAZ). Department of Physiological Chemistry, Karolinska Institutet, Stockholm, Sweden (OR, AW).

Leukotriene B_4 (LTB_4) is a potent pro-inflammatory compound, wich is formed by successive conversion of arachidonic acid by the 5-lipoxygenase enzyme and LTA_4-hydrolase. LTB_4 has been implicated in the pathogenisis of skin inflammation, particularly in psoriasis. However, the cellular origin of LTB_4 in the lesional skin has remained controversial. Recently we have shown that neutrophil derived LTA_4 can be transformed into LTB_4 by cultured keratinocytes. The purpose of this study was therefore to determine whether normal human epidermis could produce LTB_4 from LTA_4 ex vivo, and to localize this LTA_4 hydrolase activity. Epidermis was obtained by suction blister technique, and trypsinized keratinocytes were cultured in low calcium keratinocytes growth medium. The keratinocytes and the epidermis were either incubated with neutrophils or subfractionated and then incubated with free LTA_4. LTB_4 formation was determined by RP-HPLC and RIA. Western blot analysis for LTA_4-hydrolase were also carried out with two different polyclonal antisera. Epidermis incubated with human neutrophils, resulted in a 54 % increase in LTB_4 formation when compared to neutrophils incubated alone. Furthermore it was shown that human epidermis has the capacity to transform exogenous LTA_4 into LTB_4, and that this reaction obeyed Michaelis-Menten kinetics with an apparant K_m of 6 μM and a V_{max} of 150 pmol/0.5 mg protein/min. Subcellular fractionation of homogenized epidermis localized the LTA_4-hydrolase activity mainly in the 105,000 g supernatant fraction (cytoplasmic fraction). This activity was inhibited by two inhibitors of LTA_4 hydrolase (bestatin and captopril). Western blot analysis of the 105,000 g fraction of homogenized epidermis and cultured keratinocytes supported the presence of a LTA_4-hydrolase. Thus normal human epidermis possesses LTA_4 hydrolase activity which can transform exogenous LTA_4 and neutrophil-derived LTA_4 into LTB_4. The identification of LTA_4-hydrolase in the cytoplasmic fraction of human epidermis indicates that epidermal cells may play an active role in the biosynthesis of LTB_4, a potent mediator of skin inflammation. Because LTA_4 hydrolase is a key enzyme in the enzymatic cascade leading to LTB_4 formation, modulation of epidermal LTA_4 hydrolase activity may be important in inflammatory skin diseases.

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NOVEL HISTAMINE RELEASING AUTOANTIBODY IN CHRONIC URTICARIA DISTINCT FROM ANTI-FcεRI (α-SUBUNIT) ANTIBODIES

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We have recently reported that sera of many chronic urticaria patients contain histamine releasing activity on basophils of healthy volunteers which is mainly attributable to IgG autoantibodies against either the α-subunit of the high affinity IgE receptor (FcεRIα) or IgE. However, we also found that some patients' sera contained autoantibodies with a novel type of histamine releasing activity. Both whole plasma and purified IgG induced histamine release from anti-IgE responsive basophils of a specific donor. Samples from at least one patient induced serotonin secretion from the rat basophilic leukaemia (RBL-2H3) cell line, which does not respond to anti-FcεRIα or anti-IgE autoantibodies. The autoantibody-induced secretion from RBL-2H3 cells was impaired if the cells were sensitized with IgE, suggesting interaction with FcεRI. This hypothesis was further supported by synergistic enhancement of the secretion from RBL-2H3 cells by an adenosine receptor agonist (NECA), inhibition by dexamethasone pretreatment of the cells, and enhancement by pretreatment with cholera toxin, but not with pertussis toxin. However, neither histamine release from human basophils nor serotonin secretion from RBL-2H3 cells was affected by pre-incubation of patients' plasma or purified IgG with the recombinant extracellular fragment of FcεRIα up to 1 μg/ml. Furthermore, passive sensitization with myeloma IgE or serum of the donor, whose basophils were responsive to the autoantibody, did not induce responsiveness of basophils from a donor with low serum IgE. These results suggest the presence of novel types of autoantibodies that induce histamine release by the activation of FcεRI but without direct interaction with IgE or FcεRIα. Further investigation of the molecules targeted by this autoantibody may reveal a novel mechanism of mast cell activation in chronic urticaria and possibly other diseases involving mast cell activation.

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EXPRESSION OF THE SERINE PROTEINASE INHIBITOR SKALP/ELAFIN IS RELATED TO THE DEGREE OF DIFFERENTIATION OF SQUAMOUS CELL CARCINOMA. Hans A.C. Alkemade, Henri O.F. Molhuizen, Ivonne M.J.J. van Vlijmen-Willems, Urbaan J.G.M. van Haelst and Joost Schalkwijk. Depts. of Dermatology and Pathology, University Hospital Nijmegen, The Netherlands.

Skin-derived antileukoproteinase (SKALP)/Elafin is a serine proteinase inhibitor that specifically inhibits elastase and proteinase 3 with high affinity. SKALP/elafin is expressed in lesional psoriatic epidermis and in epidermis following injury, but not in normal epidermis.

Recently, we demonstrated a differential expression of SKALP/elafin in several types of human epidermal tumors. Since a relation between SKALP/elafin expression and extent of differentiation of the tumor cells was suggested, we investigated its expression in a large number of squamous cell carcinomas (SCC) using immunohistology and *in situ* hybridization.

1. At the protein level, well-differentiated SCC showed generally the highest expression; moderately-differentiated SCC showed SKALP/elafin expression in parts of the tumor fields that showed keratinization, and in the cells showing individual keratinization as well. In poorly-differentiated (parts of the) SCC, no SKALP/elafin was demonstrated using immunohistology.

2. At the mRNA level these findings were confirmed by non-radioactive *in situ* hybridization.

SKALP/Elafin may interfere with proteolytic activity of tumor cells or infiltrating inflammatory cells, and we hypothesize that within the spectrum of SCC with varying degrees of differentiation, a progressive loss of SKALP/elafin expression could facilitate tumor invasion.

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PROCESSING OF CATHEPSINS B AND L BY ASPARTIC PROTEINASES FROM RAT EPIDERMIS. A Kawada*, K.Hara**, T.Matsuyama***, K.Fukuyama, and A.Ishibashi*, Dapartment of Dermatology, University of California, San Francisco,CA, *National Defense Medical College, **Nagasaki University, and ***Tokyo University.

Cathepsins B and L, lysosomal cysteine proteinases, are synthesized as precursor forms and processed to the mature forms by aspartic proteinases. The action of epidermal aspartic proteinases on the processing of procathepsins B and L was investigated. Proteins extracted from 2-day-old rat epidermis in 20 mM sodium phosphate buffer, pH 7.0, were fractionated with ammonium sulfate and applied to an S-Sepharose Fast Flow column. Three peaks of epidermal aspartic proteinases (cathepsins E, D_1, D_2) eluted had hemoglobin hydrolyzing activity, which was inhibited by pepstatin A. Proteins with Z-Phe-Arg-MCA hydrolytic activity eluted in 0.18 M and 0.38 M NaCl contatined proforms which was immunoblotted by anti-cathepsins B and L IgG. The two fractions were incubated with each (9.2 mU of hemoglobin hydrolyzing activity) of 3 aspartic proteinases at 20°C for 12 h. Procathepsin L was processed to mature form by cathepsin D_2, but not by D_1 or E, while procathepsin B was converted to mature form by cathepsin D_1. These findings indicate that different cathepsin D isozymes in rat epidermis initially processed procathepsins B and L to mature form.

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ANTILEUKOPROTEASE IN HUMAN KERATINOCYTE DERIVED CELL LINES. Oliver Wiedow, Reinhard Kulke, and Enno Christophers, Department of Dermatology, University of Kiel, Kiel, Germany

Potent inhibitors of human leukocyte elastase such as elafin and antileukoprotease can be extracted from psoriatic scale material. Since keratinocytes are known producers of elafin, we were interested whether they may also be the source of antileukoprotease in human skin. We tested the squamous carcinoma cell line A431 and the basal cell carcinoma cell line KB for their capability to produce protease inhibitors. The A431 cell line was grown in Dulbecco's modified Eagle medium and the KB cell line in Minimum Essential Medium, both supplemented with 10% FCS. Subconfluent cultures were washed three times with PBS and further cultured in essentially protease inhibitor free medium for 24 h under standard culture conditions. Culture supernatants contained inhibitors for human leukocyte elastase and cathepsin G, whereas the lysed cells did only contain HLE inhibitory activity, but no cathepsin G inhibitory activity. The identity of the inhibitor released by the A431 and KB cell lines with antileukoprotease could be further substantiated by the use of an antileukoprotease ELISA. It could be shown that A431 and KB cells contain and secrete antileukoprotease immunoreactivity. This imunoreactivity coelutes with the HLE plus cathepsin G inhibitory activity in reversed phase HPLC. Therefore, we conclude that antileukoprotease is present in keratinocyte derived cell lines supporting the assumption that antileukoprotease in human skin is derived from keratinocytes.

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PURIFICATION AND MOLECULAR CHARACTERIZATION OF SKALP/ELAFIN, AN ELASTASE INHIBITOR WITH TRANSGLUTAMINASE SUBSTRATE MOTIFS. Henri O.F. Molhuizen, Hans A.C. Alkemade, Patrick L.J.M. Zeeuwen, Gijs J. de Jongh, Bé Wieringa*, and Joost Schalkwijk, Department of Dermatology, Academic Hospital, and the *Department of Cell Biology and Histology, Faculty of Medicine, University of Nijmegen, Nijmegen, The Netherlands.

SKALP (skin-derived antileukoproteinase), also termed elafin, is a proteinase inhibitor found in psoriatic epidermis as a short polypeptide of 6 kDa. A larger molecule with the same biochemical characteristics as SKALP/elafin was shown to be present in cultured human keratinocytes. Purification and N-terminal sequencing of this molecule and the cloning of its cDNA revealed the existence of a 117 amino acid precursor molecule. Cleavage of an hydrophobic signal sequence of 22 amino acids results in a 9.9 kDa mature protein (95 amino acids). This mature protein appeared to contain a domain with putative transglutaminase substrate motifs in addition to the known proteinase inhibiting domain of SKALP/elafin. Three different stratagies were followed to show that this domain can act as a functional transglutaminase substrate. The results clearly show that SKALP can indeed become crosslinked by transglutaminase to proteins extracted from psoriatic scales. Based on these results we speculate that SKALP/elafin, secreted by epidermal keratinocytes in psoriatic skin, can exist both as a free 6 kDa form, mainly consisting of the proteinase inhibiting domain, and as a 9.9 kDa form, covalently attached to the cornified envelopes by transglutaminase crosslinking.

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CELL:MATRIX INTERACTIONS DIRECT COLLAGENASE AND STROMELYSIN EXPRESSION BY KERATINOCYTES ACTIVELY INVOLVED IN WOUND HEALING. H.G. Welgus, U. Saarialho-Kere, S. Kovacs, A. Pentland, and W.C. Parks, Dermatology Divs., Jewish Hosp. and Washington Univ. School of Med., St. Louis, MO

Interstitial collagenase (c'ase) is the principal mammalian enzyme that cleaves insoluble type I collagen. Stromelysin, another metalloproteinase (mp), degrades various matrix components including proteoglycans, laminin, and fibronectin. Using in situ hybridization, we found that c'ase mRNA was prominently expressed by basal keratinocytes bordering the sites of active healing associated with re-epithelialization of ulcerative lesions of diverse etiologies. C'ase production was most intense in migrating epidermal cells closest to the ulcer edge. Weak signal for c'ase mRNA was seen in occasional dermal fibroblasts, but no signal was ever found in normal epidermis or non-ulcerative lesions. Stromelysin was also expressed by migrating basal keratinocytes, but a strong signal was also detected in dermal fibroblasts. TIMP, an inhibitor of mp's, was produced by perivascular and stromal cells, but away from sites of c'ase or stromelysin expression. This distinct localization suggests that keratinocyte-derived mp's act without impedance from TIMP. As demonstrated by immunostaining for type IV collagen, mp-positive keratinocytes were not in contact with an intact basement membrane and were probably migrating over the dermal matrix. These observations suggest that induction of keratinocyte mp's is directed by cell:matrix contact. Indeed, keratinocytes cultured on Matrigel did not product c'ase whereas cells grown on type I collagen expressed high enzyme levels. We hypothesize that migrating keratinocytes actively involved in re-epithelialization acquire a matrix-degradative phenotype upon contact with the dermal matrix.

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DIHYDROXYVITAMIN D3 INDUCES A RISE IN INTRACELLULAR CALCIUM IN HUMAN KERATINOCYTES, BUT VITAMIN D3 DOES NOT. K.T. Jones, P.S. Friedmann, G.R. Sharpe, Department of Dermatology, University of Liverpool, UK

Normal human keratinocytes (HKs) are induced to differentiate by 1α,25 dihydroxycholecalciferol (DHCC), the active form of vitamin D_3, whereas cholecalciferol (CC), the inactive precursor, does not have this effect. Both millimolar extracellular calcium (Cao) and the phorbol ester TPA, which induce HK differentiation, cause a sustained rise in intracellular free calcium (Cai) (1); hence Cai appears to be an important signal for differentiation. We have therefore compared the effect of both DHCC and CC with TPA on HK morphology, growth and Cai.

HKs were cultured in serum-free medium MCDB153 (70μM Cao). Cell number was measured using a Coulter Counter and growth expressed as a percentage of control cultures. Cai measurements were made on individual cells using the calcium sensitive dye Fura-2 and an inverted fluorescent microscope connected to a photon counting system.

TPA (16nM), after 1 day, induced morphological changes with the development of fusiform cells; similar effects occurred with DHCC (100nM) after 3 days but not with CC. TPA (3nM) reduced cell growth to 17±3% (mean±SEM, n=12) at 3 days. HKs were more sensitive to the growth inhibitory effects of DHCC than CC. Growth was inhibited to 56±3% and 38±3% of control with 30nM and 100nM DHCC respectively at 4 days but only 89±4% and 77±2% with CC. Although in repeated experiments no immediate Cai rise was observed with DHCC a slower increase was observed over hours with both DHCC (100nM) and TPA (16nM). For TPA Cai rose from 95±3nM to 123±3nM and 133±2nM at 6h and 24h respectively (n=50) and for DHCC to 123±4nM and 136±4nM. No such rises were observed with CC.

Both TPA and DHCC alter HK morphology, reduce growth and raise Cai. In contrast the biologically inactive CC has only a moderate effect on growth. DHCC, in common with other known differentiation stimuli, therefore appears to give a sustained rise in Cai.

1. Sharpe GR et al. Arch Dermatol Res 284:445-450, 1993

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1,25 DIHYDROXYVITAMIN D3 (CALCITRIOL) INDUCES HYPERPROLIFERATION IN MURINE EPIDERMIS WITHOUT AFFECTING EXPRESSION OF THE DIFFERENTIATION-ASSOCIATED KERATIN K1. C Lützow-Holm[1], A Heyden[1], HS Huitfeldt[2], P Brandtzaeg[1], OPF Clausen[1]. Inst. of Pathology, Univ. of Oslo[1], Rikshospitalet, Oslo, Norway., Natl. Inst. of Public Health[2], Oslo, Norway.

Vitamin D3 (calcitriol) and its analogues are anti-proliferative and promote differentiation in vitro, possibly reflecting modes of action of these drugs in psoriasis. We wanted to assess if calcitriol has a similar effect on differentiation in vivo by studying the onset of keratin K1 expression in a cohort of 5-bromodeoxyuridine- (BrdUrd-) labeled cells in hairless mouse epidermis. Epidermis was stimulated by topical application of a single dose of 0.75 nmol calcitriol. After 16 h, all proliferating S-phase cells were pulse-labeled with BrdUrd by intraperitoneal injection. Groups of four animals were sacrificed at intervals up to 48 h following BrdUrd injection. The labeling index (LI) and keratin K1 expression of the postmitotic cell cohort were revealed simultaneously by paired immunofluorescence staining. Calcitriol induced proliferation as shown by a 100% increase in LI 17 h after its application. Suprabasal expression of K1 occurred in the BrdUrd-positive cells 18-24 h after BrdUrd injection, similar to that seen in vehicle treated epidermis. The unchanged onset of keratin K1 expression supports the notion that calcitriol does not specifically modulate the keratin K1 expression but rather influences the keratinocyte differentiation program through other genomic or non-genomic signals.

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THE LOCALIZATION AND REGULATION OF CRABP mRNA EXPRESSION IN HUMAN SKIN: EFFECTS OF AGING, CELLULAR DIFFERENTIATION, AND MALIGNANT TRANSFORMATION. Mark S. Eller, Scot G. McAfee, Dan Harkness, Peter Muz, Mina Yaar, Gary S. Rogers and Barbara A. Gilchrest. Department of Dermatology, Boston University School of Medicine, Boston, MA

The cellular retinoic acid-binding proteins (CRABPs) I and II are thought to modulate the concentration of retinoic acid reaching the nuclear retinoic acid receptors. CRABP II is the isoform predominately expressed in human skin and in cultured skin cells, at least at the mRNA level. To examine the level and distribution of CRABP mRNAs in vivo, in situ hybridization of skin sections with CRABP riboprobes was performed using anti-sense in vitro transcripts to hybridize to mRNAs, and the sense transcripts as negative controls. CRABP II antisense probes hybridized strongly to newborn foreskin suprabasal keratinocytes, while sections from adult skin showed variable hybridization. However, this message was consistently expressed at high levels in the more differentiated, inner root sheath cells of hair follicles in adult skin. Basal keratinocytes from all donors contained minimal levels of CRABP II mRNA. Basal and squamous cell carcinomas also expressed little if any CRABP II mRNA while the epidermis adjacent to or overlying these tumors showed hybridization comparable to newborn foreskin epidermis. The overall pattern of CRABP II mRNA expression in skin was found to be similar to that for SPR1, also called pancornulin, a protein thought to be involved in cornified envelope formation during keratinocyte differentiation. In situ analysis with CRABP I riboprobe failed to detect this message in normal skin but did show hybridization to cells in the papillary dermis surrounding both basal and squamous cell carcinomas. These combined data support a role for CRABP II in keratinocyte differentiation. Furthermore, CRABPs I and II may be modulated in vivo by aging, sun exposure and/or malignant transformation. The absence of CRABP II in skin cancers and the association of this protein with the differentiated keratinocyte phenotype suggest that the loss of CRABP II may be causally related to carcinogenesis.

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EXPRESSION OF C-KIT LIGAND IN HUMAN KERATINOCYTES. Eishin Morita, Dong Geun Lee*, Masanori Sugiyama*, Shoso Yamamoto Department of Dermatology, *Department of Gene Engineering, Hiroshima University School of Medicine, Hiroshima, Japan

c-kit ligand (also known as stem cell factor) is expressed on tissue-anchored stromal cells, and plays an important role in the development of c-kit- bearing cells, such as hematopoietic cells, germ cells, mast cells and melanocytes. In the present study, we investigated whether human keratinocytes are able to express c-kit ligand.

mRNA for c-kit ligand was detected by using the reverse transcriptase-mediated polymerase chain reaction (PCR) technique with specific primers. Primers were designed to amplify an 882bp fragment from full length-type cDNA and a 798bp fragment from spliced type cDNA. DNA sequencing of the PCR-amplified DNA was carried out by using the dideoxy method.

A cDNA fragment with the size of approximately 880bp was amplified by PCR from cDNA preparation of cultured keratinocytes. The DNA sequence of this fragment was identical to the corresponding part of the previously-reported cDNA sequence of c-kit ligand. mRNA for c-kit ligand was also detected in epidermis that was freshly obtained by the suction blister technique. This result indicates the spontaneous transcription of full length-type mRNA of c-kit ligand in human keratinocytes.

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COMPLEXES OF MULTIPLE TRANSCRIPTION FACTORS IN REGULATION OF KERATIN SYNTHESIS. Irwin M. Freedberg, Mamitaro Ohtsuki, Thierry Magnaldo, Francoise Bernerd, Ruben Vidal and Miroslav Blumenberg, Ronald O. Perelman Department of Dermatology, NYU Medical Center, New York, USA

A common feature of all epithelial cells is the keratin intermediate filament cytoskeletal network. Whereas other cell types often use a specific master transcription factor to coordinate cell type specific transcription (e.g. Myo D in muscle cells), analysis of transcriptional regulation of keratin genes suggests that specific groupings of widely expressed transcription factors, acting on clusters of recognition elements in the promoter regions, confer epithelia-specific transcription. We have defined such a cluster of three sites that binds five transcription factors in the human K#5 keratin gene, which is expressed in basal cells. Within this cluster an unusual Sp1 site binds the Sp1 transcription factor and two additional proteins. Flanking the Sp1 site are an AP2 site and another sequence, Site A, each binding a single transcription factor. Activated keratinocytes express K#16. Within the K#16 gene promoter we have identified a cluster that binds the same five specific proteins. Somewhat further upstream an EGF responsive element binds its associated protein. Another tight cluster of protein binding sites binds three different proteins, including transcription factor AP-2. Deletion of any of the sites completely abolishes or severely reduces promoter activity, without extinguishing it totally. Similar clusters of recognition sites for the same five transcription factors have been identified in other keratin genes as well and we believe they play a role in epithelia-specific expression of keratins.

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REGULATION OF P53 SYNTHESIS AND NUCLEAR LOCALIZATION IN NORMAL AND TRANSFORMED KERATINOCYTES. M.K. Kuechle and M.R. Pittelkow, Department of Dermatology, Mayo Clinic, Rochester, MN USA

The wild type p53 (wt p53) gene product is a negative nuclear growth regulator in selected cell types. Mutations of p53 induce uncontrolled proliferation and promote cellular transformation and immortalization. p53 mutations appear to be involved in early or later events in carcinogenesis. Inactivation or degradation of wt p53 may occur by complexing with viral oncoproteins E6 (HPV 16, 18) and SV40 large T. We investigated expression and protein localization of p53 in epidermis, cultured keratinocytes and epithelial cells. We also examined effects of UV or γ radiation on growth arrest and p53 nuclear localization since these environmental factors are capable of inducing inhibition of DNA synthesis and mutagenic events. Monoclonal and polyclonal antibodies MAb 421, 122, DO-1, DO-7, 1801 and CM-1 were used to detect and localize p53 protein. Cultured keratinocytes showed both cytoplasmic and nuclear reactivity with selected antibodies, indicating expression of wt p53 and localization to specific subcellular compartments during the cell cycle. p53 protein also was expressed by normal epidermis and cultured keratinocytes and synthesis was confirmed by metabolic labelling with ^{35}S-met and immunprecipitation. The estimated half-life of p53 in cultured keratinocytes was 2-3 hours. UV and γ irradiation induced marked nuclear localization of p53 in cultured keratinocytes and epidermis that preceded decrease in PCNA expression, BrdU incorporation and G_1 growth arrest in cultured keratinocytes. These studies demonstrate that p53 is expressed by normal epidermis and keratinocytes, has a longer half-life than found in mesenchymal cells and becomes localized to the nucleus prior to UV and γ radiation induced-inhibition of DNA synthesis and growth arrest. wt p53 may exert significant biological activity in normal growth control and the protection of epidermal keratinocytes from unrepaired mutagenic damage.

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PANUCORNULINS BELONG TO AN EPIDERMAL AND EPITHELIAL MULTIGENE FAMILY. Mary Ann Greco, William Lane, Deborah J. Ladas and Howard P. Baden, Cutaneous Biology Research Center, Massachusetts General Hospital, Charlestown, MA, USA

Pancornulins, small basic proteins associated with epidermal differentiation, were originally identified by crossreaction to a cornified envelope (CE) derived monoclonal antibody. Analysis of amino acid sequence indicates that these proteins are members of the spr-1 (small proline rich) multigene family, cDNAs originally identified by subtractive analysis of UVC irradiated keratinocytes. The 14.9 kDa pancornulin is identical to the protein deduced from spr-1 clone 128 by mass spectroscopy and partial amino acid sequence analysis. The 16.9 kDa protein shares 92% and the 22 kDa 76% identity with the clone. These proteins have unusually high proline, glutamine, lysine and cysteine contents. A decreased level of the 22 kDa pancornulin was observed after growing cells in media low in vitamin A, while cells maintained in media low in calcium and then switched to normal calcium showed increased pancornulin protein and mRNA levels. The 14.9 kDa pancornulin was upregulated by UVC exposure similar to spr-1, but the 16.9 kDa and 22 kDa proteins were more dramatically induced. Amino acid sequence unique to the 22 kDa pancornulin was used to generate primers to reverse transcribe and PCR amplify total keratinocyte mRNA. The PCR products (~220-250 bp) were subcloned and sequenced. Like the protein deduced from spr-1 clone 128, the amino acid motif VPEPC*PK was present as a repeat in these partial cDNAs, but a number of amino acid substitutions were predicted in these clones. The findings that pancornulins are able to act as amino donors and acceptors and are part of a multigene family suggest that the proteins act as bridge molecules in CE formation.

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MULTIPLE ISOFORMS OF THE HEAT SHOCK PROTEIN HSP27 AND THEIR UNIQUE PATTERNS OF SUBCELLULAR LOCALIZATION IN HUMAN KERATINOCYTES. Rivkah Isseroff, Linda M. Lombard, and Marla McClaren, Dept of Dermatology, Univ of Calif Davis School of Medicine, Davis, CA.

The small heat shock protein, HSP27, is ubiquitously expressed and though its function is not clear, is believed to play a role in both growth control and the acquisition of thermotolerance. We have previously demonstrated that HSP27 is expressed in unstressed human keratinocytes and relocates to the Triton-insoluble cytoskeleton and the nucleus after either heat or chemical (arsenite) stress. Four isoforms of HSP27 have been demonstrated in other cells after stress, and here we report the isoforms expressed in human keratinocytes. Using an antibody to HSP27 and Western blotting of cultured keratinocyte lysates separated either by 2-D gel electrophoresis, or by 1 dimensional isoelectric focussing, we find 2 major isoforms expressed in unstressed cells, 8 in heat-shocked cells, and 5 in arsenite-stressed cells. Subcellular fractionation reveals that specific isoforms localize to the nuclear, cytosolic, membrane and cytoskeletal subcellular compartments. Additionally, we identify the cytoskeletal component to which HSP27 binds as actin: HSP27 is co-precipitated by anti-actin antibody immunoprecipitation of cell lysates, and co-localizes with phalloidin-stained filaments by confocal microscopy of fixed, cultured cells. The association of individual isoforms with specific subcellular components suggests that the posttranslational modifications of HSP27 may direct their ultimate cellular localizations, and may help us determine the function of these ubiquitously expressed proteins.

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A ROLE FOR KERATINOCYTE-DERIVED IL-10 IN THE INDUCTION OF TOLERANCE IN CUTANEOUS IMMUNE RESPONSES. A. H. Enk*, V. L. Angeloni, M. C. Udey and S. I. Katz, *Dept. of Dermatol. Univers. of Mainz, F.R.G. and Dermatol. Branch, NCI, NIH, Bethesda, U.S.A.

Inhibition of T cell-derived IFN-g and IL-2 production by IL-10 occurs via an indirect mechanism that is dependent on the presence of viable APCs. We have recently demonstrated that murine keratinocytes (KC) produce IL-10, and sought to determine if KC-derived IL-10 could regulate immune responses in skin by modulating Langerhans cell (LC) function. Initial studies indicated that exposure of BALB/c LC to IL-10 in vitro did not increase or decrease MHC class II antigen expression of LC. Production of IFN-g and IL-2 (but not IL-6) by stimulated T cells was significantly reduced however (> 50%). More dramatic results were obtained when the H-2k-derived cytochrome c-specific Th1 clone AE7 was studied. Exposure of LC to IL-10 (100U/ml) for 24h prior to initiation of cultures with AE7 cells and intact protein antigen (or the relevant peptide antigen) resulted in virtually complete inhibition (> 90%) of proliferation and cytokine production. In marked contrast, IL-10-treated and sham-treated LC supported proliferation of the H-2k-derived conalbumin-specific Th2 clone D10.G4 equally well. Because dendritic cells are reportedly resistant to the effects of IL-10, we also tested the ability of IL-10 to influence cultured as compared with freshly-prepared LC. The negative influence of IL-10 on proliferation of Th1 cells was only observed when fresh or 24h cultured LC were used; exposure of 48-72h cultured LC to IL-10 did not inhibit their accessory cell activity. To determine if AE7 cells exposed to antigen in the presence of IL-10-pretreated LC were unresponsive to subsequent stimulation, T cells were recovered after 24h of coculture with IL-10-pretreated LC and antigen and restimulated immediately (or after a period of 1-5 days) with untreated LC in the presence of antigen. AE7 cells exposed to antigen in the presence of untreated LC responded normally to further stimulation; AE7 cells exposed to antigen in the presence of IL-10-pretreated LC were anergic, but remained IL-2 responsive. Our data suggest that keratinocyte-derived IL-10 plays an important role in the regulation of cutaneous immune responses in skin and implicates epidermal LC as key intermediaries.

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PLATELET-DERIVED EOSINOPHIL-CHEMOTACTIC CYTOKINE RANTES. Yoshikazu Kameyoshi, Jens-M. Schröder+, Enno Christophers+, Shoso Yamamoto, Department of Dermatology, Hiroshima University School of Medicine, Hiroshima, Japan and +Department of Dermatology, University of Kiel, Kiel, Germany.

Eosinophil(Eo)-dominant infiltration of inflammatory cells suggests the existence of factors which attract Eos preferentially. Well described chemotaxins such as C5a or PAF attract neutrophils as well. Recently we have identified Eo-selective chemotactic activity in supernatants of thrombin-stimulated platelets. This activity was attributed to 8kD polypeptide(s), suggesting that this factor could belong to the IL-8-related chemokine family. Accordingly, we have further characterized this Eo-chemotaxin. By the use of HPLC techniques, two Eo-chemotactic peptides for (EoCP-1 and 2) were purified to homogeneity. NH2-terminal amino acid sequence analyses revealed identical sequences for both EoCPs, and these were identical with cytokine RANTES. The molecular weight(MW) of EoCP-2, determined by electrospray mass spectrometry, was 7862.8±1.1, which agreed well with the calculated value of RANTES. The observed MW of EoCP-1 was 8355±10, suggesting a glycosylated form of RANTES. Both EoCPs showed potent chemotactic activity for Eos (ED50=2nM), but no significant activity for neutrophils. Specific desensitization experiments indicated that RANTES binds to different receptors from those for C5a or PAF. Eo-selective chemotactic activity of RANTES was confirmed using recombinant material. It is conceivable that cytokine RANTES plays an important role in Eo-infiltration in inflammation.

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A DENDRITIC CELL LINE CAPABLE OF INDUCING CONTACT SENSITIVITY REACTIONS AND SENSITIVE TO UVB RADIATION. G. Girolomoni[1], P. Paglia[2], F. Granucci[2], F. Robbiati[3], A. Giannetti[1], and P. Ricciardi-Castagnoli[2]. [1]Dept. of Dermatology, University of Modena; [2]CNR, Center of Cytopharmacology, Milano; [3]Lepetit Research Center, MMDRI, Gerenzano, ITALY.

Dendritic cells (DC) are potent antigen presenting cells in the activation of unprimed T cells and in the induction of primary T cell-mediated immune responses. DC, however, are difficult to isolate from tissues and can be maintained in culture only for short period of time. In this study, we show for the first time that functional DC lines can be obtained from spleen primary cultures using the novel retroviral vector, MIB-ψN11. One clone, named CB1, displayed most of the features described for DC, including DC morphology, constitutive expression of B7/BB1 molecule, and reactivity with several anti-DC specific mAb. CB1 cells potently stimulated allogeneic T cells in the primary mixed leukocyte reaction. GM-CSF was required to increase the low constitutive MHC class II expression and for effective presentation of myoglobin to antigen-specific hybridoma. CB1 cells pulsed *in vitro* with haptens (DNBS or FITC) and injected s.c. into naive syngeneic mice were able to induce contact sensitivity (CS) responses. The ability of CB1 cells to induce CS was increased (>50-80%) by prior treatment with GM-CSF, but markedly inhibited by paraformaldehyde fixation or by exposure to UVB radiation (0-400 J/m^2) immediately after hapten coupling. The results indicate that cloned DC represent a useful model to study the antigen presenting cell functions of DC and to investigate the effects of UVB radiation on DC.

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CHARACTERIZATION OF CD45 EXPRESSED ON HUMAN LANGERHANS CELLS. EVIDENCE FOR DYNAMIC SPLICING AND FUNCTIONAL INTERFERENCE WITH THE HIGH AFFINITY RECEPTOR FOR IGE. Thomas Bieber[1,2], Mareen Jürgens[1], Andreas Wollenberg[1], Daniel Hanau[2], and Henri de la Salle[2]. [1]Dept. of Dermatology, University of Munich, Germany and [2]Laboratoire d'Histocompatibilité, CRTS, Strasbourg, France.

Depending on the cell type and its maturation/functional stage, the extracellular region of the CD45/tyrosine phosphatase undergoes a complex splicing involving various combinations of exons 4, 5 and 6 (also named A, B, and C) producing distinct isoforms. Although it is well known that human Langerhans cells (LC) express CD45, as yet, clear data about the isoform(s) and the function are missing. This was the aim of the present study. In a first approach, RNA was extracted from highly purified LC and examined for the presence of transcripts for CD45 by PCR-amplification. Amplification fragments were then identified by DNA-sequencing and restriction analysis. Thereby, freshly isolated LC expressed transcripts lacking all 3 exons (CD45R0). In short time cultured LC, transcripts including only the exon 5 (CD45RB) rapidly appeared while CD45RO remained present. Double labeling experiments with flow cytometric analysis using antibodies against various isoforms of CD45 confirmed that freshly isolated LC are predominantly CD45R0 while CD45RB rapidly emerged in culture. Since it has been reported that cross-linking of CD45 leads to unresponsiveness of anti-IgE stimulated basophils, we tested whether CD45 may interfere with the IgE-mediated activation of LC from atopic individuals. While cross-linking of FcεRI on freshly isolated LC lead to a Ca^{++}-mobilization, the response of freshly isolated LC preincubated with anti-CD45 antibody was dramatically decreased. Hence, in LC, CD45 is subjected to a dynamic splicing most likely of functional importance in terms of FcεRI-mediated activation.

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GRANULOCYTE / MACROPHAGE COLONY STIMULATING FACTOR IS AN INTRINSIC KERATINOCYTE-DERIVED GROWTH FACTOR FOR HUMAN MELANOCYTES IN UVA MELANOGENESIS. Genji Imokawa, Yukihiro Yada, Mitsutoshi Kimura, and Naoko Morisaki, Kao Biological Science Laboratories, Tochigi, Japan

We recently demonstrated that endothelins (ET) secreted from human keratinocytes (HK) is an intrinsic mitogen for human melanocytes (HM) in UVB melanogenesis (Imokawa, et al, J.Bio.Chem. 266, 18352,1993). We show here that UVA melanogenesis is associated with other HK-derived growth factors which are specifically stimulated to secrete after exposure of HK to UVA light. The medium conditioned by UVA-exposed HK elicited a significant increase in DNA synthesis by cultured HM in a UVA dose-dependent manner, which was associated with no increase in the intracellular calcium level upon incubation with HM. Analysis of ET-1 and IL-1α, major HK-derived cytokines involved in UVB-associated HM proliferation, revealed that UVA light did not cause HK to stimulate the secretion of the two cytokines. By contrast, several cytokines such as IL-6, IL-8 and granulocyte / macrophage colony stimulating factor (GM-CSF) significantly increased in the conditioning medium of HK after exposure to 1.0 J/cm^2 of UVA light. Gel chromatographic profile of HK conditioning medium demonstrated that there were two different mitogenic factors (P-1 and P-2) with molecular weights of approximately <1,000 and 20,000, respectively, with larger one (P-1) also effective in increasing melanization as measured by ^{14}C-thiouracil. The quantitation of cytokines through the chromatographic fractions by ELISA revealed that the active fraction (P-1) was consistent in the profile of molecular weight with that determined for GM-CSF. Furthermore, the stimulatory effect on DNA synthesis of HM by the P-2 fraction was completely neutralized by the addition of antibodies for GM-CSF, but not for bFGF and SCF. These studies suggest that GM-CSF secreted by HK play an essential role in the maintenance of HM proliferation and UVA hyperpigmentation in epidermis.

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HB-EGF, A NEW MEMBER OF EGF FAMILY, IS AN AUTOCRINE GROWTH FACTOR FOR NORMAL HUMAN KERATINOCYTES. Koji Hashimoto,[1] Shigeki Higashiyama,[2] Hideo Asada,[1] Teruaki Kobayashi[1] and Kunihiko Yoshikawa,[1] [1]Department of Dermatology and [2]Department of Biochemistry, Osaka University School of Medicine, Osaka, Japan

Heparin-binding EGF-like growth factor (HB-EGF) is a new member of EGF family purified from conditioned medium of the U-937 macrophage-like cell line. Since HB-EGF shares similarity to amphiregulin, an autocrine growth factor for human keratinocytes (NHK), we investigated whether HB-EGF is an autocrine growth factor for NHK. NHK were cultured in serum-free MCDB 153 medium. We tested recombinant HB-EGF for its ability to stimulate NHK in the absence of exogenous TGF-α and EGF. Optimal stiumlation of growth occured at 0.5 and 1.0 ng/ml (1.8 fold increase in cell number). To examine the production and secretion of HB-EGF by NHK, we partially purified HB-EGF from NHK condition medium by heparin-affinity cloumn. Western blot showed a 22 kd band comigrating with HB-EGF purified from U-937 cell conditioned medium. Northern blot analysis detected a single major 2.5-kb transcript of HB-EGF in NHK. Next, we examined auto-induction of HB-EGF mRNA in NHK. Addition of HB-EGF at 20 ng/ml increased HB-EGF mRNA 2.3 fold and TGF-α mRNA 6.6 fold after 6 h. Interestingly, addition of TGF-α and EGF enhanced HB-EGF mRNA 1.8 fold and 2.0 fold, respectively, at 6 h. These results demonstrate that HB-EGF is an autocrine growth factor for NHK. Furthermore, HB-EGF and TGF-α stimulate their synthesis interactively, suggesting the presence of complex loop system for autocrine growth factor production in NHK.

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THE STRUCTURE OF HUMAN TRICHOHYALIN AND ITS EXPRESSION IN BACTERIAL SYSTEMS: S.-C. Lee, I.-G. Kim and P.M. Steinert, Skin Biology Branch, NIAMS; and S.-I. Chung, Laboratory of Cellular Oncolcoy and Development, NIDR, NIH, Bethesda, Maryland 20892, U.S.A.

Trichohyalin (THH) associates in regular arrays with the keratin intermediate filaments (KIF) of the inner root sheath and medulla cells of the hair follicle, filiform papillae of the tongue, and the granular layer of terminally differentiating epidermis. While it is also known to be a major substrate of the calcium-dependent transglutaminase and peptidylarginine deiminase enzymes, little is known about its structure and precise function(s). We have determined the full-length coding sequence of human THH by use of RNA-mediated anchored PCR methods and from a genomic clone. Analysis of its secondary structure reveals at least three important functions in these cells. The protein of about 250 kDa is unusual in that it contains the highest content of charged residues of any protein known in biology. Of several well-defined domains, domains 2,3,4,6 and 8 are almost entirely α-helical, configured as a series of peptide repeats of varying regularity, and are predicted to form a single-stranded α-helical rod stabilized by ionic interactions between charged residues on successive turns of the α-helix. Domain 6 is the most regular and may bind KIF directly by ionic interactions. Domains 5 and 7 are less regular and may introduce folds. Thus THH is predicted to form an elongated flexible rod at least 220 nm long. THH may function in part as a KIF associated protein by linking the KIF in loose flexible networks. Secondly, THH is structurally similar to, but several times longer than, involucrin (45 nm), a known cell envelope constituent. Thus, by crosslinking with transglutaminases, together THH and involucrin may serve as scaffold proteins in the organization of the cell envelope of these cells, and perhaps anchor the cell envelope to the KIF. Finally, THH possesses a pair of functional EF-hand-type calcium binding domains on its amino-terminus, which may be involved in its own calcium-dependent postsynthetic processing during terminal differentiation. The availability of full-length cDNA and genomic clones will permit detailed studies on each of these functions in normal and pathological tissues. To initiate this work, we have now expressed the long α-helical domains of THH in the pET 11a bacterial expression system and purified the expressed proteins from inclusion bodies. These will be used for in vitro binding assays with various types of KIF and as substrates for the known epidermal transglutaminases and peptidyl arginine deiminiase.

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COMPLETE CHARACTERIZATION OF THE HUMAN TYPE VII COLLAGEN GENE. Jouni Uitto, Angela M. Christiano, Linda C. Chung-Honet, and *Daniel S. Greenspan. Department of Dermatology, Jefferson Medical College, Philadelphia, PA; and *Department of Laboratory Medicine, University of Wisconsin, Madison, WI.

We recently demonstrated strong genetic linkage between the dystrophic forms of EB and the gene for human type VII collagen (COL7A1) on chromosome 3p21. To facilitate elucidation of the underlying mutations in EB patients, we initiated extensive cloning of the corresponding gene and cDNA. Deduced amino acid sequences of the ~9.2 kb mRNA revealed that the α1(VII) chain consists of a central collagenous domain characterized by repeating Gly-X-Y sequences which contain 22 imperfections, flanked on the 5'-side by a large non-collagenous domain (NC-1), and on the 3'-side by a smaller non-collagenous (NC-2) domain. The chimeric organization of NC-1 revealed modules with homology to cartilage matrix protein (CMP), nine consecutive fibronectin type III domains, and the A domain of von Willebrand factor. These domains with homology to other adhesive proteins may contribute to the interaction of the NC-1 domain with other components of the basement membrane and anchoring plaques. The corresponding gene is remarkably complex, containing more exons than any previously published gene, and is unusually compact, particularly for a collagen gene. The COL7A1 gene encompasses 118 exons in a region of ~32 kb of genomic DNA, leading to an intron/exon ration of 2.4:1. Elucidation of the structure of the COL7A1 gene has facilitated the identification of mutations in patients with recessive dystrophic EB, and provides the basis for the design of gene replacement therapy in these patients.

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EPIDERMAL GROWTH FACTOR (EGF)-INDUCED ENHANCEMENT OF HUMAN SQUAMOUS CARCINOMA CELL MIGRATION ON TYPE I COLLAGEN INVOLVES SELECTIVE UPREGULATION OF $\alpha_2\beta_1$ INTEGRIN EXPRESSION Kimio Fujii and Sadao Imamura, Department of Dermatology, Kyoto University Faculty of Medicine, Kyoto, Japan

Some human epithelial tumors overexpress EGF receptor and the degree of expression is correlated with their invasive phenotype. Since tumor cell invasion into surrounding stroma involves integrin-mediated cell-matrix interactions, the effects of exogenous EGF on cell-matrix interactions were compared between normal human keratinocytes and HSC-1 human cutaneous squamous carcinoma cells overexpressing EGF receptor by cell adhesion and cell migration assays. Pretreatment of normal keratinocytes with 5-50 ng/ml EGF for 18 hr did not influence the cell adhesion on fibronectin, fibrinogen, laminin or type I collagen (I). The same treament of the HSC-1 cells selectively enhanced the cell migration and adhesion on I. Pretreatment with 50 ng/ml EGF increased the number of spread cells and the size of migratory area on I by 250% and 400%, respectively, but not those on other substratum. ELISA and immunoprecipitation studies showed that EGF upregulated the expression of $\alpha_2\beta_1$ integrin by 4 fold increase, but not that of $\alpha_3\beta_1$, $\alpha_5\beta_1$ or $\alpha_v\beta_3$ integrin, suggesting that selective upregulation of $\alpha_2\beta_1$ collagen receptor is involved in the EGF-induced enhancement of the carcinoma cell interactions with type I collagen. This may be relevant to the advantageous role of EGF receptor overexpression to the invasive phenotype of epithelial tumor cells.

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MOLECULAR MECHANISM OF CD8β GENE EXPRESSION. Yasuhiro Kawachi, Fujio Otsuka, Department of Dermatology, Institute of Clinical Medical Science, University of Tsukuba, Tsukuba, Japan

It is known that CD8 molecule plays an important role in immune response of T cells. We identified a regulatory region of the mouse CD8 β-chain gene (CD8β) promoter. The CD8β 5' upstream sequence could not drive the expression of the bacterial chloramphenicol acetyltransferase (CAT) gene without T cell receptor or SV40 enhancer elements. The results of transient transfection assays indicated that the dominant transcription-activating element within the CD8β-promoter is located at -45 to -40bp (CCGCCC) from the transcriptional initiation site. Elimination of this element, by deletion or specific point mutation, significantly reduced transcriptional activity from this promoter. The sequence of this core region corresponds to a GC box motif which is known to act as a binding site for a uniquitously expressed transcriptional activator, Sp1. However the promoter activity appeared to be T cell specific, and the gel retardation assay using the core sequence as a probe revealed formation of complexes with multiple nuclear factors, one of them being specific to T lineage cells. These data suggest that the CD8β promoter requires a cis-acting element as well as several nuclear factors for full-range, tissue-specific transcription.

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DELETION OF TATA PROMOTER REGION IN A HUMAN K17 GENE FRAGMENT CLONED BY PCR. Paul Bowden and Ronald Marks, Department of Dermatology, University of Wales College of Medicine, Cardiff, UK.

Keratin gene expression is tightly controlled during normal epidermal differentiation and is aberrant in skin disorders characterised by abnormal keratinisation. Cloning and sequencing of keratin genes has shown that a single mutation can be responsible for significant skin pathology. K17 responds to alterations in epidermal homeostasis, and like K6 & K16, shows increased expression in keratinisation disorders. The K17 gene responds to UVR and to cytokines making regulation of HK17 complex. We have recently cloned two HK17 genes and PCR amplified parts of HK17 from human genomic DNA. One of the PCR amplified fragments was found to have a 56 bp deletion of the TATA box promoter region, rendering the gene inactive.

Synthetic oligonucleotides containing different restriction enzyme sites (Sst I and Xba I) were made, one to 5'non-coding sequences of HK17 (300 bp upstream) and the other to exon 1 sequences. PCR amplification of type I keratin cosmid clones and genomic DNA produced a fragment of about 700 bp, which in some cases was a doublet. The amplified genomic DNA was ligated into pGEM-3Z, cloned and sequenced. One cloned DNA fragment was 694 bp in length and covered the 5'non-coding and exon 1 sequences of HK17. The exon 1 sequences were 97% homologous to the cDNA but comparisons with HK17 gene sequences showed only 88% homology due to discordance at the 5'end. Comparison with HK17 pseudogene sequences showed that the cloned PCR fragment had no TATA box due to a 56 bp deletion over the promoter region. As neither of the described HK17 pseudogenes contain a promoter deletion, this clone probably represents a third HK17 pseudogene. Whether this gene exists in the keratin clusters defined on chromosome 17, or elsewhere in the genome, remains to be seen.

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REGULATION OF THE SYNDECAN FAMILY OF MATRIX AND GROWTH FACTOR CO-RECEPTORS. DETECTION OF AN INDUCTIVE ACTIVITY RELEASED DURING WOUND REPAIR. R.L. Gallo, C. Kim, M. Ono, M. Klagsbrun, C. Page, E. Eriksson, and M. Bernfield Harvard Medical School, Boston, MA

The syndecans are a family of 4 cell surface heparan sulfate (HS) proteoglycans that bind growth factors and extracellular matrix components and can control the interaction of these effectors with their classical receptors. This interaction and control indicates the syndecans act as co-receptors. Prior studies have shown syndecan-1 induction in dermal tissue during wound repair, thus suggesting a potential function during this process. We found induction of both syndecan-1 and -4, but not syndecan-2, in the dermis of murine skin wounds. Because of this induction, we evaluated wound fluid (WF) for inductive activity. Cell surface syndecans were measured by ELISA and western blot and mRNA was evaluated by Northern blot in NIH 3T3 cells. Early wound fluid caused a ca. 3-fold induction of syndecan-1 and ca. 10-fold induction of syndecan-4 but no change in syndecan-2 at the cell surface. No induction was seen after exposure to other biological fluids and growth factors (fetal calf or porcine serum, chick or mouse embryo extract, amniotic fluid, late phase wound fluid, TNFα, TGFα, EGF, TGFβ, IGF-1, IGF-2, IL-1, IL-3, IL-4, IL-6, GM-CSF). Cell surface induction was accompanied by increased mRNA, cell surface stability, and decreased glycosylation. A variety of mesenchymal cells, but not epithelial cells, were induced. Characterization of the inductive activity revealed it to be heat labile, >50 kDa, not heparin-bound, stable to thiols and acid, and eluted from a C4 reverse phase HPLC at ca. 29% acetonitrile. Therefore, a previously unrecognized factor(s) released during wound repair regulates syndecan-1 and -4 expression specifically in mesenchymal cells. Induction of these co-receptors provides a mechanism for dermal mesenchymal cells to integrate matrix and growth factors during wound repair.

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REGULATION OF CONNECTIVE TISSUE GROWTH FACTOR GENE EXPRESSION BY TRANSFORMING GROWTH FACTOR-BETA. Hitoshi Okochi, Nobukazu Hayashi*, Yasumasa Ishibashi, Gary R. Grotendorst*. Department of Dermatology, University of Tokyo, Tokyo, Japan. *Department of Cell biology & Anatomy, University of Miami, FL, U.S.A.

Connective tissue growth factor (CTGF) is a cysteine rich peptide that exhibits platelet-derived growth factor-like biological and immunological activities. We previously demonstrated that transforming growth factor beta (TGF-ß) induced high levels of CTGF mRNA and protein in human skin fibroblasts. In this study we investigated the regulation of CTGF gene expression by TGF-ß. We have isolated the human CTGF gene and made CTGF promoter-luciferase constructs by placing the CTGF promoter nucleotides -823 to +74 into the pGL2-basic vector. Luciferase assays revealed that TGF-ß treated NIH/3T3 fibroblasts exhibited a 10.5 fold induction of luciferase activity compared with non-treated cells after 24 hours incubation. Other growth factors such as PDGF or FGF stimulated a 2-3 fold induction. We find that the CTGF promoter is responsive to TGF-ß in human skin fibroblasts and NIH/3T3 fibroblasts, but not cos7 cells, an epithelial cell line. These results suggest that TGF-ß regulation of CTGF gene expression is tissue specific. Deletion mutants analysis indicates that the TGF-ß regulatory elements are located between position -276 to -128 of the CTGF promoter sequence.

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VITAMIN D RECEPTOR mRNA IS NORMAL IN LESIONAL PSORIATIC SKIN. Karsten Fogh, Morten Svendsen, Anders Åström, Henrik Sølvsten, and Knud Kragballe, Department of Dermatology, Marselisborg Hospital, University of Aarhus, Aarhus, Denmark.

Treatment with analogs of vitamin D_3 improves psoriasis. Vitamin D_3 is believed to mediate its actions by binding to the intracellular vitamin D receptor (VDR). The purpose of the present study was, therefore, to study the expression of the VDR in normal and psoriatic skin. Total RNA was extracted from normal human skin, uninvolved and involved psoriatic skin by use of cesiumchloride gradient centrifugation. Methods for determination of mRNA included Northern analysis and ribonuclease protection assay (RPA). As a control parallel experiments were performed using cDNA for the cellular retinoic acid binding protein type II (CRABP-II). ^{32}P-labelled detection probes for Northerns were generated using cDNAs for VDR and CRABP-II as templates, whereas ^{35}P-labelled detection probes for RPA were synthezed by in vitro reverse transcription. By Northern analysis, VDR-mRNA was not detectable in any of the tissues analyzed, whereas CRABP-II-mRNA was markedly increased in lesional psoriatic skin. By use of RPA, VDR-mRNA was readily detected in both normal and lesional skin. There was no difference in the levels of VDR-mRNA when comparing normal human skin, uninvolved and involved psoriatic skin. Also by RPA, CRABP-II-mRNA was increased in the involved psoriatic skin compared to normal human skin and uninvolved psoriatic skin. The results show that the expression of the VDR (as measured by mRNA) is normal in lesional psoriatic skin. However, to further study the function and the regulation of the VDR in the skin, it remains to be determined whether VDR protein is altered in the skin of psoriasis.

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PROLACTIN AND PROLACTIN RECEPTOR AND THEIR GENE EXPRESSION IN THE HUMAN ECCRINE SWEAT GLAND. G.Soos, F. Sato, and K. Sato, Marshall Dermatology Research Labs, Univ. of Iowa College of Medicine, Iowa City, IA, USA.

Although the eccrine sweat gland is one of the largest cutaneous appendages, detailed knowledge of its mechanisms and the communication between the sweat gland and the rest of the skin are still poorly understood. The roles of prolactin (PRL) in immunomodulation, growth control, regulation of membrane transport and the antagonism between PRL and cyclosporin A (CsA) are well known. PRL-like immunoreactivity was previously described in the eccrine sweat gland. In an attempt to clarify the potential role of PRL in skin/sweat gland, we investigated the presence of PRL and PRL receptors (PRLR) in human sweat glands, fibroblasts, and keratinocytes. Western blot analysis of clean human sweat showed PRL-immunoreactive bands at 55-68 KD Amino-methylcoumarin acetic acid-conjugated PRL bound most prominently to the luminal membrane of the duct, suggesting that PRL may be synthesized in the sweat gland and binds to receptors located there. Total RNA was extracted from the epidermal sheet, cultured subconfluent human sweat coil and duct cells, foreskin fibroblasts, and keratinocytes and subjected to Northern blot and reverse-transcription polymerase chain reactions (RT-PCR). While mRNA for PRL is present in the sweat gland cells, fibroblasts and keratinocytes, mRNA for PRLR was detected most in the duct and secretory cells and to a lesser extent in keratinocytes. PRL stimulated interleukin-6 gene expression in sweat gland cells in a dose dependent manner. We conclude that PRL is produced and PRLR is expressed in the skin as they are abundantly present in sweat glands and in sweat while also detectable in keratinocytes. PRL may act like an autocrine hormone in the regulation of ductal function, either directly or mediated by IL-6, an antagonist of IL-8 gene expression in the sweat gland. Furthermore, the known antagonism between CsA and PRL and the expression of the latter in normal keratinocytes, sweat glands and fibroblasts suggest that CsA treatment in some pathological conditions might interfere with altered PRL production in these cells.

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RESTRICTED AND HAIR CYCLE-DEPENDENT EXPRESSION OF CLASSICAL AND NON-CLASSICAL MHC CLASS I ANTIGENS IN NORMAL MOUSE SKIN. Stefan Eichmüller, Ralf Paus, Udo Hofmann, Peter Robinson* and Beate M. Czarnetzki. Dpt. of Dermatology, University Hospital R. Virchow, Freie Universität Berlin, Germany, *CRC Transplantation Biology Section, Harrow, Middlesex, U.K.

Except for some cells in the growing rat and human hair follicle, all nucleated cells in mammalian skin are believed to express classical MHC class I antigens (MHC I). Here, we report the first immunohistological profile of classical and non-classical MHC I expression in the skin of adolescent C 57 BL-6 mice during all stages of the depilation-induced hair cycle. We demonstrate the absence of MHC I (H-2b, H-2Db) immunoreactivity (ir) in the proximal hair bulb of growing (=anagen) hair follicles and analyze its hair cycle-dependent intrafollicular distribution. In anagen VI follicles, keratinocytes of the hair bulb matrix and inner root sheath are strikingly H-2b negative, while all other cells, including dermal papilla fibroblasts label H-2b+. In contrast, the dermal papilla of regressing (=catagen) and resting (=telogen) follicles is H-2b negative (due to antigen masking by extracellular matrix molecules?). In addition, we present the first evidence of non-classical MHC I antigen expression in normal skin. During all hair cycle stages, the distal hair bulb shows strong Qa-2 ir in the periinfundibular region. Qa-2+ keratinocytes are also H-2b+ and are restricted to an epithelial follicle compartment containing gamma-delta T cells (cf. Hofmann et al., this meeting). This raises intriguing questions as to the "immune privilege" of the hair follicle and to the role of H-2b and Qa-2 molecules in both hair growth and skin immune responses. We suggest the murine hair cycle as a fascinating, yet virtually unexplored model for studying the functions of classical and non-classical MHC I molecules in developmentally regulated tissue-interaction systems.

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CULTURED HUMAN HAIR FOLLICLE PROGENITOR CELLS ARE DISTINCTIVE. Amanda Reynolds & Colin Jahoda, Dept. Biological Sciences, Durham University, U.K.

Hair follicle stem cells are potentially amongst the most accessible in the adult body and the recognition, isolation and evaluation of these cells represent important steps towards an understanding of skin and hair biology. Recently, rat vibrissa follicle germinative cells have been isolated and interest has been generated in the label-retaining cells of the upper follicle "bulge". Neither cell type has been isolated from human follicles, with most previous work involving epidermal cell culture from plucked hair fibres. In contrast, our strategy was to investigate the "protected", less differentiated cells which are left behind after plucking. Germinative cells were microdissected from human follicle end bulbs using fine forceps. While it was not feasible to precisely delineate the human follicle "bulge", mid/upper basal outer root sheath ("bulge-region") cells could be isolated and cultured. These two cell types were studied in isolation and in recombination with follicle dermal papilla cells, and compared with epidermal cells from plucked fibres and the basal region of interfollicular skin. The study involved at least ten repetitions of each variation, in minimal essential medium, antibiotics and either 20% fetal calf serum or epidermal supplements. In isolation, the germinative cells displayed a unique small, round phenotype and remained inactive. By contrast, cell counts and repeated passaging revealed that the "bulge-region" cells were more proliferative and viable than those from plucked tissue or interfollicular skin. In association with papilla cells, germinative and "bulge-region" cells participated in complex interactive behaviour, not seen in other recombinations. In particular, the germinative/papilla cells formed composite organotypic structures containing impressively intact basement membrane at the dermal-epidermal interface, as well as the hair-specific form - glassy membrane. In summary, human hair follicle germinative and "bulge-region" epidermal progenitor cells display intriguingly distinctive properties and their availability provides considerable scope for study within and beyond the hair follicle.

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A COMPARISION OF GROWTH AND ANDROGEN RECEPTOR CONTENT OF DERMAL PAPILLA CELLS FROM BALDING AND NON-BALDING HUMAN SCALP FOLLICLES. Hibberts N.A & Randall V.A. Dept of Biomedical Sciences, University of Bradford, U.K.

Androgens gradually transform large terminal hairs in some scalp regions of genetically susceptible people to small vellus ones resulting in baldness. The mechanisms behind this are unknown, although androgens are believed to act on the mainly epithelial hair follicles via the mesenchyme derived dermal papilla. We are comparing androgen action in balding scalp hair follicles with non-balding ones in an attempt to understand this process.

Dermal papillae were microdissected from balding and non-balding scalp follicles and cultured in various media. Cell growth was assessed by haemocytometer counting of 2.5 x 10^4 cells in 35mm tissue culture dishes over 15 days. Androgen receptor content was measured by incubation of confluent cells in 100mm dishes for 2 hours at 37 C in media containing 10% charcoal stripped serum with ^{3}H-mibolerone (0.05-10nM;10 points) +/- 100x excess unlabelled mibolerone to determine non-specific binding.

Dermal papilla cells from balding (n=3) and non-balding (n=3) scalp grew about 3x faster in media containing human serum. Saturable androgen receptors were present in dermal papilla cells from balding (Kd=0.236 nmol/l; n=5) and non-balding scalp (Kd=0.300 nmol/l; n=4). However the androgen receptor content of balding scalp dermal papilla cells (Bmax=0.058 fmol/10^4 cells; n=5) was higher than non-balding (Bmax=0.039 fmol /10^4 cells; n=4). No difference in androgen receptor specificity was found between balding (n=5) and non-balding (n=4) dermal papilla cells.

The presence of higher levels of specific saturable androgen receptors in cultured dermal papilla cells from balding scalp follicles sugest that they may form a useful model for further studies into how androgens inhibit terminal hairs.

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ANALYSIS OF THE TUMOR NECROSIS FACTOR (TNF)α RESPONSIVE REGION OF THE HUMAN ICAM-1 GENE. L-J Li, S Naik, L Cornelius, Y Xu, T Nguyen, N Shibagaki, J Bauer, RA Swerlick, TJ Lawley, SW Caughman. Department of Dermatology, Emory University School of Medicine, Atlanta, GA, USA.

ICAM-1 is an adhesion molecule critical in leukocyte binding to human dermal microvascular endothelial cells (HDMEC). It is rapidly upregulated on HDMEC and HMEC-1, an immortalized HDMEC line, upon exposure to TNFα. To investigate the molecular mechanisms involved in TNFα-induced enhancement of ICAM-1 expression, we have generated and transfected into HDMEC and HMEC-1 a sequential series of 5', internal, and 3' deletion ICAM-1-based CAT reporter gene constructs. The effects of TNFα (100 U/ml for 16 h) upon transfectant lysate CAT activity were then determined. Cells transfected with a series of constructs from -1162/+1 (transcription start site=+1) through -384/+1 retained a TNFα responsiveness of 10-fold enhanced CAT activity when compared to untreated controls. However, with construct -277/+1 only a 3-fold increase was observed, and constructs from -182 or below displayed no TNFα-responsiveness. Potential regulatory regions defined by these studies (-384/-182 and -277/-182) were used as radiolabelled probes in gel mobility shift assays using nuclear protein extracts from either untreated or TNFα-treated HDMEC. While shift complexes were seen with probe -277/-182 which could be competitively inhibited with excess specific but not irrelevant DNA, these complexes were also seen with control extracts. However, with probe -384/-182, a specific complex appeared only with extracts from TNFα-treated cells, and this complex could be competitively inhibited only with excess cold specific DNA. This region does not contain a consensus NFκB binding sequence, the *cis*-element normally implicated in TNFα-induced enhanced transcription. Thus, TNFα-induced upregulation of ICAM-1 is transcriptionally mediated and involves an induced or activated *trans*-acting protein which specifically interacts with a DNA element located between bp -384 and -277 of the ICAM-1 5' transcriptional regulatory region.

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PHORBOL ESTER STIMULATION OF HUMAN DERMAL MICROVASCULAR ENDOTHELIAL CELLS IS ASSOCIATED WITH INCREASED TOPOISOMERASE-1 ACTIVITY. Helen A Bull & Pauline M Dowd. Division of Dermatology, Department of Medicine, UCLMS, London UK.

Antibodies to the enzyme topoisomerase-1 (topo-1)(anti-Scl-70 antibodies) are a feature of scleroderma and sera from patients with antibodies to topo-1 inhibit bovine topo-1 enzyme activity in vitro. Topo-1 catalyses DNA unwinding and hence regulates DNA replication and transcription and cellular differentiation. Scleroderma is characterised by microvascular changes, however, it is not known whether topo-1 is involved in signal transduction pathways associated with the differentiation of microvascular endothelium or whether antibodies to topo-1 can block cellular differentiation. We have therefore investigated topo-1 activity in phorbol ester stimulated human dermal microvascular endothelial cells (HDMEC).

Confluent monolayers of HDMEC were stimulated with 0.5 μM phorbol myristate acetate (PMA) for up to 28 hours and at different timepoints following stimulation individual flasks of cells were lysed with 0.5% NP40. The lysates were spun; the supernatant was used for Western blot analysis with a polyclonal anti-topo-1 antiserum and the nuclear pellet was assayed for topo-1 enzyme activity using ϕX174 RF DNA (Form 1) as a substrate.

Topo-1 enzyme activity was detected in the nuclear extract of unstimulated HDMEC and in HDMEC stimulated with 0.5μM PMA there was a rapid time-dependent increase in activity which was maximal at 1 hour following stimulation and still elevated at 28 hours. The polyclonal anti-topo-1 antiserum detected a band with M_r 70 kDa on Western blots of unstimulated HDMEC. PMA stimulation of HDMEC resulted in a rapid loss of the immunoreactive M_r 70 kDa band which was evident after only 10 min stimulation with PMA and maintained up to 28 hours.

These results demonstate that PMA stimulation of HDMEC is associated with increased topo-1 enzyme activity and a decrease in immunoreactive protein. The increase in topo-1 activity indicates the potential role for this enzyme in signal transduction pathways mediating HDMEC differentiation. Antibodies to topo-1 may well block such signal transduction pathways thus precipitating the microvascular endothelial cell changes associated with scleroderma.

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PRENATAL DIAGNOSIS OF OCULOCUTANEOUS ALBINISM BY TYROSINASE GENE ANALYSIS. Hiroshi Shimizu[1], Hironori Niizeki[1], Kaoru Suzumori[2], Ryoji Aozaki[3], Ryuji Kawaguchi[3], Kazumasa Hikiji[3] and Takeji Nishikawa[1]. [1]Department of Dermatology, Keio University School of Medicine, Tokyo, [2]Department of Obstetrics and Gynecology, Nagoya City University Medical School, Nagoya, and [3]Genetic Research Laboratory, SRL, Tokyo, Japan.

Tyrosinase-negative oculocutaneous albinism (OCA), an autosomal recessive trait, is caused by tyrosinase gene mutations. Until now, prenatal diagnosis of this condition has been made only by fetal skin biopsy. For the first time, we succeeded in making a prenatal diagnosis of OCA by tyrosinase gene analysis of the genomic DNA of the fetal cells. A pregnant Japanese woman, the mother of a patient, a boy with tyrosinase-negative OCA, desired a prenatal diagnosis. The presence of pathological mutation sites of tyrosinase gene were studied in the patient and his family members using PCR-amplification and allele-specific oligonucleotide hybridization. The patient was confirmed to be mutant homozygote, having insertion of C residue in the exon 2 that shifts the reading frame and introduces a premature termination signal (TGA codon) after the amino acid residue 298. His parents, grandmothers, but not grandfathers were confirmed to be heterozygote. For prenatal diagnosis, fetal cells were obtained by amniocentesis at 14 week gestation and the fetal genomic DNA was studied by the same method. The result clearly demonstrated that the fetus was heterozygote and not affected. We believe this technique is safe, less invasive, and can provide quick and reliable prenatal diagnosis of tyrosinase negative OCA in an earlier stage of pregnancy.

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MOLECULAR ANALYSIS OF A TYROSINASE-NEGATIVE ALBINISM. Kyoung C. Park, Chaya D. Chintamaneni, Ruth Halaban, Carl J. Witkop, Jr., Byoung S. Kwon

The individuals with tyrosinase-negative oculocutaneous albinism were studied for the detection of mutation. Sequence analysis of the tyrosinase coding region from an individual with tyrosinase-nagative oculocutaneous albinism revealed that the patient was a compound heterozygote. One allele carried a C to A single base substitution at codon 355 of exon 3, and the other carried two nucleotides deletion in exon 1. The nucleotide substitution caused a putative amino acid change from threonine(ACA) to lysine(AAA), abolishing a signal for N-glycosylation. The two base pair deletion caused a frameshift, creating a putative premature termination signal at codon 226. The melanocyte from the proband and her affected brother were amelanotic and devoid of measurable tyrosinase activity. Moreover, gel electrophoresis analysis of the immunoprecipitated proband tyrosinase showed that the protein was not processed to the mature glycosylated form, confirming the predicted consequence of the amino acid change. The two base deletion on the heterologous allele was detected only by sequencing genomic DNA. The transcript of this allele was nor represented in the cDNA library and could not be detected by PCR mRNA, and the putative truncated protein was not present in immunoprecipitates, suggesting that the allele with the missense mutation may be preferentially expressed.

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IDENTIFICATION OF MUTATIONS IN THE *FACC* GENE. MU Udono[1], PC Verlander[1], JD Lin[1], RA Gibson[2], CG Matthew[2], and AD Auerbach[1]. 1. The Rockefeller University, New York, NY, USA. 2. Div. Med. & Mol. Genetics, UMDS Guy's Hospital, London, U.K.

Fanconi anemia (FA) is a rare autosomal recessive disorder in the family of DNA repair disorders that include Bloom's syndrome, ataxia telangiectasia, and xeroderma pigmentosum. FA is defined by the sensitivity of cells to the effects of DNA crosslinking agents such as diepoxybutane, and is clinically associated with a spectrum of congenital malformations that may include radial aplasia, hyperpigmentation, and short stature, among others. FA patients eventually develop progressive pancytopenia and are predisposed to cancer, especially acute myelogenous leukemia. A cDNA has recently been cloned (*FACC*) that corrects the crosslink sensitivity of FA complementation group C cell lines.

We are using single strand conformation polymorphism (SSCP) analysis to screen genomic DNA from affected individuals for mutations in *FACC*. Individuals representing 174 families in the International Fanconi Anemia Registry (IFAR) are currently being screened, and six different bands with altered migration have been identified thus far in a total of 22 different families. Two of these shifted bands appear to represent polymorphisms rather than mutations based on the segregation of the aberrant band in the five families in which they were found. The most recently identified aberrant band, in exon 4, has been found in ten of eleven Jewish families and in two other families. In each of these families, the affected individuals are homozygous for the band shift, while the unaffected individuals are either heterozygous or are homozygous for the normal band. The presence of this mutation in the Jewish population of the IFAR suggests a possible founder effect.

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RANDOM X-CHROMOSOME INACTIVATION OF PURIFIED CELLS DERIVED FROM LANGERHANS CELL HISTIOCYTOSIS TISSUES - EVIDENCE AGAINST CLONAL PROLIFERATION OF INVOLVED CELL TYPE. Raymond Yu, ^Carol Chu, *Laki Buluwela and Anthony Chu. Unit of Dermatology, Royal Postgraduate Medical School, *Dept of Biochemistry, Charing Cross and Westminster Hospital, London, and ^Duncan Guthrie Institute of Medical Genetics, Glasgow, U.K.

Langerhans cell histiocytosis (LCH) is a condition characterised by an abnormal accumulation and/or proliferation of cells expressing the phenotype of normal epidermal Langerhans cells (LC). The cause of LCH is unknown, some speculate LCH may represent a neoplastic transformation of LC or its progenitor cell. In this study, we obtained tissues from 3 female patients affected by LCH, genomic DNA extracted from purified cells was used for PCR based X-chromosome inactivation assays. Cell purification procedures with either immunomagnetic beads or FACsorting were used to separate CD1a-positive from CD1a-negative cells. PCR amplification using the oligoprimers flanking HpaII sites and the trinucleotide repeats of the Human Androgen Receptor was performed on genomic DNA derived from the purified cells with or without prior Hpa II digestion. Our results demonstrated the heterozygous status of the three female LCH sufferers and controls. DNA pre-digested with HpaII from the LCH patients showed equal band intensity in both CD1a+ and CD1a- populations. Our data demonstrated that the LCH cells from these patients exhibit random X-chromosome inactivation and strongly argue against clonal proliferation of LCH cells.

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DIFFERENTIAL REGULATION OF 180KD AND 230KD BULLOUS PEMPHIGOID ANTIGENS IN THE FORMATION AND DISINTEGRATION OF HEMIDESMOSOMES. Yasuo Kitajima, Katsushi Owaribe[1], Yoshiaki Hirako[1], M.Koji Owada[2] and Hideo Yaoita, Dept of Dermatol, Jichi Med School, Tochigi, Japan, [1]Dept of Mol Biol, School of Sci, Nagoya Univ, Nagoya, Japan, [2]Inst of Mol & Cellul Biol for Pharmaceut Sci, Kyoto Pharmaceut Univ, Kyoto, Japan

To clarify the mechanisms of hemidesmosome formation, we studied the rearrangement of the 180kD and 230kD bullous pemphigoid antigens(BPAs) by low(0.07mM)-high(1.87mM) Ca^{2+} shift and 12-tetradecanoylphorbol-13-acetate(TPA) treatment, and their TPA-induced phosphorylation in cultured keratinocytes(DJM-1) by immunological methods using monoclonal antibodies to these BPAs. The 180kD BPA was distributed on the whole cell surface in paraformaldehyde-fixed cells. Pretreatment with 0.5% Triton X-100(TrX) erased the antigen from the non-ventral membrane, but retained that on the ventral membrane. The 230kD BPA was distributed in the cytoplasm and on the ventral surface without TrX treatment, and only on the ventral membrane after TrX treatment. Since TrX does not solublize the proteins bound to keratin intermediate filaments(KIFs), but does those free from KIFs, it is suggested that the 180kD BPA on the non-ventral surface is drifting in the cell membrane, the 230kD BPA is pooled in the cytoplasm, and both BPAs on the ventral membrane are bound to KIFs. In low Ca^{2+} cells, both BPAs on the ventral cell membrane were distributed evenly as fine dots, whereas they formed a concentric ring or arch pattern in high Ca^{2+}cells as studied by immunofluorescence. TPA (16nM), which activated protein kinase C, disrupted transiently(1-2) the ring pattern. TPA treatment(15min) caused a profound increase in phosphorylation (serine) and an increase (1kD) in molecular weight of the 180kD BPA on immunoprecipitation and SDS-PAGE. Phosphorylation of the 230kD BPA was not detected. These data suggest that these two BPAs are differentially controlled to form hemidesmosomes on the ventral membrane.

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DEVELOPMENT OF AN ELISA TO DETECT ANTI-BP180 AUTOANTIBODIES IN THE SERA OF BULLOUS PEMPHIGOID AND HERPES GESTATIONIS PATIENTS. L.A. Diaz, K. Wilske, A. Taylor, D.J. Emery, and G.J. Giudice Dept. of Dermatology, Medical College of Wisconsin and VAMC, Milwaukee, WI, USA

One of the primary antigenic targets of autoantibodies produced by bullous pemphigoid (BP) and herpes gestationis (HG) patients is a 180 kD hemidesmosomal protein, BP180. Our laboratory has recently cloned the BP180 antigen and has identified a dominant epitope recognized by BP and HG autoantibodies. In this investigation we have developed an ELISA system to detect autoantibody reactivity against this cloned epitope. A 42 amino acid segment of the BP180 NC16A domain has been expressed in E. coli as a glutathione S-transferase fusion protein (GST-BP180-SΔ1) using the pGEX expression system. An affinity-purified preparation of GST-BP180-SΔ1 (1.7μg) was immobilized on microtiter wells and incubated with a 1:100 dilution of the following sera: BP (n:62), HG (n:28), endemic pemphigus foliaceus (EPF)(n:17), lupus erythematosus (LE)(n:15) and normal human sera (NHS)(n:22). Microtiter wells coated with identical amounts of recombinant GST were used as a negative control. Bound autoantibodies were labeled with a horseradish peroxidase-conjugated goat-anti-human Ig. The chromogenic product was measured at 492 nm. Overall, 35 of 62 BP sera (56%) and 18 of 28 HG sera (64%) showed specific reactivity in this assay. Specific autoantibody activity in both sets of patients was predominantly of the IgG class (no IgM, IgA or IgE was detected). None of the control sera (EPF, LE or NHS) were positive in this assay. These results confirmed and extended a previous finding from our laboratory that BP and HG sera recognize a common antigenic site on the BP180 ectodomain. Based on its high sensitivity (56 to 64%) and specificity (99.7%) for detecting BP and HG autoantibody activity, the ELISA assay described here may prove to be a valuable diagnostic tool.

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BULLOUS PEMPHIGOID AND HERPES GESTATIONIS AUTOANTIBODIES RECOGNIZE A COMMON NON-COLLAGENOUS SITE ON THE BP180 ECTODOMAIN. G.J. Giudice, D.J. Emery, B.D. Zelickson, G.J. Anhalt, Z. Liu, and L.A. Diaz, Depts of Dermatology, Med. Coll. of Wisconsin, Milwaukee, WI, USA; Univ. of Minnesota, Minneapolis, MN, USA; Johns Hopkins Univ. Sch. of Med., Baltimore, MD, USA and the VAMC, Milwaukee, WI

Bullous pemphigoid (BP) and herpes gestationis (HG) are skin diseases characterized by subepidermal blisters and autoantibodies against two hemidesmosomal (HD) antigens, BP230 and BP180. In the present investigation fusion proteins (FPs) encompassing various segments of the BP180 antigen were expressed in a prokaryotic system and assayed by immunoblotting and immunoadsorption against a panel of BP, HG and control sera. One antigenic site, comprising 14 amino acids of the BP180 NC16A domain, was shown to be recognized by 60% of BP sera and by 63% of HG sera tested. Immunoadsorption analysis identified this region of BP180 as an immunodominant site. Ultrastructural localization using an affinity-purified rabbit antiserum raised against a recombinant form of BP180 showed that this BP/HG autoantibody-reactive region is localized to the epidermal basal lamina immediately adjacent to the HD. These findings confirm the predicted type II transmembrane orientation of the BP180 antigen. The long, C-terminal collagenous domain projects into the basal lamina and may function as a site of interaction with an extracellular matrix component. The major non-collagenous segment of the BP180 ectodomain, NC16A, contains an immunodominant site recognized by autoantibodies from a majority of BP and HG sera. Autoantibodies directed against this site may be relevant in subepidermal blister formation in BP and HG.

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THE PRECURSOR FREQUENCY OF PERIPHERAL BLOOD LYMPHOCYTES (PBL) FROM PATIENTS WITH BULLOUS PEMPHIGOID (BP) IS INCREASED AGAINST AMPHIPATHIC SYNTHETIC PEPTIDES FROM BPAG1. MJ Rico, RD Streilein, RP Hall, Duke University and the Durham VA Medical Center , Durham, NC.

Sera from patients with BP contains autoantibodies specific for multiple epitopes on two distinct basement membrane zone proteins, and elevated IL-2 receptors. These data suggest that the autoantibody response in these patients is antigen driven, and supports a potential role for T cells in the initiation and regulation of autoantibody production. The purpose of the present study was to determine if PBL from patients with BP and controls display increased precursor frequencies against amphiphathic and hydrophilic synthetic peptides encoded by BPAG1. PBL were isolated from patients and controls and co-cultured for 7 days in the presence of 18-22 amino acid long synthetic hydrophilic (6 peptides) or amphipathic (8) peptides. 3H-thymidine uptake was determined and the precursor frequency for each peptide determined by limiting dilution analysis based on the Poisson distribution. 7/10 patients with BP and 3/10 controls demonstrated an increased precursor frequency (>1:300,000) against at least one amphipathic peptide. Minimal reactivity was noted in patients or controls against hydrophilic peptides. Increased precursor frequencies in the BP patients were noted against synthetic peptides within or adjacent to the 5' region of the coiled-coil region: 6/10 BP patients and 2/10 controls had increased precursor frequencies to peptides at the 5' end of the coiled coil region, adjacent to a region in which we have previously mapped autoantibody binding activity. This region also includes a region with sequence homology to the human heat shock protein, hsp60. These data demonstrate an increased precursor frequency in the PBL of patients with BP and in some normal controls against amphipathic peptides from BPAG1 and supports the role of T cells in the pathogenesis of BP.

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COMPARATIVE SERA REACTIVITY OF PATIENTS WITH BULLOUS PEMPHIGOID (BP) FROM JAPAN AND THE UNITED STATES (US) AGAINST FUSION PROTEINS SYNTHESIZED FROM THE COILED-COIL REGION OF BPAG1. RP Hall, T Hashimoto, K Watanabe, MJ Rico, T Nishikawa, Departments of Medicine (Dermatology) and Dermatology, Duke University Medical Center, Durham, NC, and Keio University School of Medicine, Tokyo, Japan.

Recent collaborations have demonstrated that sera from Japanese, British and US patients with BP share similar antigen profiles for reactivity with the 230 kD BPAG1. Sera from US and Japanese patients identify a major epitope on the carboxyl terminal region of BPAG1. The purpose of the present study was to determine if epitope reactivity patterns are shared between Japanese and US BP patients to fusion proteins outside of the carboxyl terminal domain. Sera from 34 Japanese patients (circulating antiBMZ titer ≥ 1:80, 16/34 reactive on immunoblot with BPAG1) and 30 US patients with BP were assayed by immunoblot for reactivity to 3 fusion proteins generated from BPAG1 cDNA: FP3 (AA1003-1193, 21 kD)from the amino end of the coiled-coil region; FP7 (AA1623-1812, 21 kD) from the carboxyl end of the coiled-coil region; and FP9 (AA683-853, 18 kD) adjacent to the 5'end of the coiled-coil region. Immunoblot reactivity was as follows (number positive/number tested):

Sera	FP3	FP7	FP9
Japanese	3/31	8/34*	1/20
United States	10/30*	10/30*	2/20
Control	0/19	1/22	2/10

*Statistically significant reactivity of US patients to FP3 and FP7 was seen when compared to controls; Japanese patients reacted to FP7 but not to FP3. These findings suggest that epitope reactivity to fusion proteins near the coiled-coil region of BPAG1 is not universally shared between Japanese and US patients with BP.

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STUDIES OF CICATRICIAL PEMPHIGOID AUTOANTIBODIES REACTIVITY ON THE IMMUNODOMINANT EPITOPE OF THE BP180 ECTODOMAIN AND NICEIN. Ph. Bernard[1], G. Giudice[2], D. Aberdam[3], J.P. Ortonne[3], L.A. Diaz[2] and C. Prost[4], Departments of Dermatology, [1] CHU Dupuytren, Limoges, France, [2] Med. Coll. Wisconsin, Milwaukee, USA, [3] CHU Pasteur, Nice, France, [4] Hôpital Saint-Louis, Paris, France.

Cicatricial pemphigoid (CP) is an autoimmune subepidermal bullous disease characterized clinically by mucous membrane involvement and scar formation and by extracellular immune deposits on the lamina densa of the dermo-epidermal junction. Recent studies indicate that CP target-antigen is probably heterogeneous, the two best candidates being BP180 autoantigen (J Invest Dermatol 1992,99,174) which is a 180 kD a hemidesmosomal protein recognized by bullous pemphigoid (BP) and herpes gestationis autoantibodies (J Invest Dermatol 1992, 99, 243), and epiligrin (J Clin Invest 1992, 90, 1628). We have studied CP sera by immunoblotting (IB) on a glutathione S-transferase fusion protein (FP-BP180-SΔ1) encompassing the dominant epitope recognized by human approximately 50 % of BP sera which is localized extracellularly within the sub-basal dense plate associated with the epidermal hemidesmosome. Sera from 20 CP patients with typical clinical and immunoelectron microscopic features were tested in this study, at a dilution of 1 : 200., using the peroxydase method. Fourteen of these CP sera recognized a 180 kDa antigen by IB on epidermal extracts. Control sera included BP sera, normal human sera and R306.1, a rabbit polyclonal antibody raised against the immunodominant epitope of the BP180 ectodomain. CP sera were also tested by IB against purified nicein/BM600 (3 subunits of 150, 125 and 100 kDa), a laminin isoform which shows similarities with epiligrin. We found that only two out of 20 CP sera recognized FP-BP180-SΔ1. These two FP-BP180-SΔ1-positive sera also demonstrated reactivity against 180 kDa antigen by IB on epidermal extracts. None of the 20 CP sera showed reactivity against purified nicein/BM600. These results suggest that the hypothetical dominant epitope recognized by CP antibodies is different from both the immunodominant epitope of BP sera and nicein/BM600, and may be located on other extracellular domain of BP180.

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IDENTIFICATION OF A BASEMENT MEMBRANE ZONE ANTIGEN REACTIVE WITH A CIRCULATING IgA ANTIBODY IN OCULAR CICATRICIAL PEMPHIGOID. EP Smith, TB Taylor, LJ Meyer, and JJ Zone, Division of Dermatology, University of Utah, Salt Lake City, UT, USA

Ocular cicatricial pemphigoid (OCP) is characterized by linear deposition of IgG and/or IgA along the basement membrane zone (BMZ) in conjunctival biopsies, but the target antigen responsible for the immune response has never been identified. We evaluated the antigenic specificity of OCP sera using Western blots against epidermal extracts and immunoaffinity purified antibodies.

Our patient selection was based on the presence of clinical evidence of ocular disease (excluding those with oral and skin disease) and a positive direct immunofluorescence (IF) of conjunctival biopsies for IgG and/or IgA.

Indirect IF revealed only IgA (not IgG) antibodies. Immunoblotting with epidermal extracts was performed using sera from OCP patients and an OCP antibody directed against a 45 kD antigen was found in all seven patients enrolled in our study. Western blots and separated skin were used as immunoaffinity substrates for purification of an IgA antigen-specific antibody. This immunoaffinity purified antibody bound to the 45 kD region on Western blot and bound in a linear fashion to the BMZ on separated skin. This IgA antibody failed to show specificity to the 230 or 180 kD bullous pemphigoid antigens and the 97 kD linear IgA bullous dermatosis antigen.

We conclude that OCP sera contain a unique IgA antibody that binds to a 45 kD BMZ antigen.

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Epitope mapping of Epidermolysis Bullosa Aquisita patient sera by molecularly cloned cDNA for type VII collagen. Toshihiro Tanaka, Fukumi Furukawa, and Sadao Imamura Department of Dermatology, Faculty of Medicine, Kyoto University, Kyoto 606, Japan.

Epidermolysis bullosa acquisita is a subepidermal blistering disease in which patients have autoantibodies against the non-collagenous domain of type VII collagen. Starting with previously isolated 1Kb cDNA for this autoantigen, we isolated overlapping cDNAs with a combined open reading frame of ~3.2Kb, encoding most (~115KDa) of the N-terminal non-collagenous domain of type VII collagen. To localize immunogenic domain, we produced the maltose binding protein fusion protein with cDNA encoding different portions of this autoantigen. Immunoblot analysis of these fusion protein with epidermolysis bullosa acquisita patient sera demonstrated that each patient serum binds to different region of this molecule and that epitopes for these patient sera locate throughout this autoantigen. These data suggest that multiple epitopes on the N-terminal non-collagenous domain of type VII collagen are recognized by circulating autoantibodies in patients with epidermolysis bullosa acquisita.

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AUTOANTIBODIES TO EPILIGRIN ARE A SPECIFIC MARKER FOR A UNIQUE FORM OF CICATRICIAL PEMPHIGOID (CP). K.B. Yancey, Z. Lazarova, G.J. Anhalt, W.R. Gammon, R.A. Briggaman, M. Welch, C. Huff, and N. Domloge-Hultsch, Bethesda, MD, Baltimore, MD, Chapel Hill, NC, Washington, DC, Denver, CO.

The major integrin ligand in cultured human keratinocyte (HK) extracellular matrix (ECM) is a glycoprotein complex called epiligrin. In human skin, epiligrin is found in the lamina lucida subregion of epidermal basement membrane (BM) where it is thought to be associated with anchoring filaments. We have identified six patients who have IgG autoantibodies that stain HK ECM, bind the superior aspect of lamina densa in 1 M NaCl split skin, and immunoprecipitate (IP) a set of disulfide-linked polypeptides in HK media, cell extracts, and ECM in the same manner as P1E1 anti-epiligrin monoclonal antibody (MAb) and MAb GB3. Studies of other patients with the same clinical phenotype (i.e., CP)(N=10) found that while seven have anti-BM autoantibodies (IgG, N=5;IgA, N=3), these autoantibodies are directed exclusively against the epidermal side of 1 M NaCl split skin, present in low titer (i.e., < 1:12), nonreactive with HK ECM, and negative in IP studies of HK media and cell extracts. These findings indicate that CP is a disease phenotype in which patient autoantibodies target different BM constituents and that anti-epiligrin autoantibodies are a specific marker for a unique form of CP that we propose to call anti-epiligrin CP.

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IMMUNOBLOT ANALYSIS OF THE CASES WITH CONCOMITANT ANTI-INTERCELLULAR AND ANTI-BASEMENT MEMBRANE ZONE IGG ANTIBODIES. Kyoko watanabe, Takashi Hashimoto, and Takeji Nishikawa, Department of Dermatology, Keio University School of Medicine, Tokyo, Japan .

We have obtained 9 sera which clearly demonstrated concurrent anti-intercellular (IC) and anti-basement membrane zone (BMZ) IgG autoantibodies with immunofluorescence of human skin section. These cases are clinically diagnosed as bullous pemphigoid (BP), herpes gestationis, BP vegetans or pemphigus foliaceus (PF). The purpose of the present study was to identify the antigens recognized by these sera with immunoblot analysis using normal human epidermal extracts (separated by either EDTA or dispase treatment) and bovine muzzle desmosome preparation. A number of possible antigen molecules were detected by these sera in various patterns; i.e., desmoplakins (DP) I/II, desmoglein (PF antigen), Pemphigus vulgaris antigen, desmocollins I/II, the 230 kD BP antigen and the 180 kD BP antigen were detected by 3, 4, 1, 1, 6, and 4 sera, respectively. One serum from BP-like case reactive with DP I/II clearly stained the intercalate disks with immunofluorescence of human and mouse cardiac muscle section, suggesting the anti-DP antibodies are responsible to its IC staining in the epidermis. These results indicate that the cases with concurrent anti-IC and anti-BMZ antibodies are highly heterogeneous and that some of them may represent the coexsistence or intermediate type of BP and pemphigus.

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ERYTHEMA MULTIFORME - DEMONSTRATION OF AUTOANTIBODIES AGAINST DESMOPLAKIN I. Dagmar Foedinger, Barbara Boecskoer, Gerhard Gruber, Klaus Wolff, Klemens Rappersberger, Department of Dermatology, Division of General Dermatology, University of Vienna Medical School, Vienna, Austria

It is generally accepted that autoantibodies play a crucial role in the etiopathogenesis of autoimmune bullous diseases. The target antigens either belong to the cadherin family of cell adhesion molecules e.g. pemphigus, or are associated with major adhesive components of the dermo-epidermal junction zone e.g. hemidesmosomes in bullous pemphigoid and anchoring fibrils in epidermolysis bullosa acquisita. 5 patients with mucocutaneous erythema multiforme (EM) with few cutaneous target lesions and widespread erosions and superficial ulcrations of the oral mucous membranes not unlike those seen in paraneoplastic pemphigus but without conjunctival lesions were studied for immunomorphological and biochemical features. Histopathology revealed dermal/epidermal EM in all 5 patients. Direct immunofluorescence showed distinct deposits of IgG in a punctated desmosomal staining pattern in 3 patients, 2 other patients showed linear IgG and IgA deposits in the basment membrane zone. Indirect immunofluorescence (IIF) on monkey esophagus revealed circulating autoantibodies in all 5 patients. These autoantibodies (IgG_1,IgA) labelled the intercellular spaces of the epithelium in a pemphigus like pattern. By IIF on mouse myocardium and mouse liver 3 sera showed binding to the desmosomal structures of both, the epithelial and non-epithelial tissue, 1 serum bound to liver desmosomes and 1 to the intercalated discs of the myocardium, respectively. IF on cultured human keratinocytes revealed a clearcut desmosomal staining pattern. Immunoblot experiments with epidermal extracts derived from normal human split skin and extracts from cultured human keratinocytes showed a distinct reaction of the circulating antibodies with a protein of approximately 250 kD that comigrated with desmoplakin I (DP I). By immunogold-immunoelectronmicroscopy the in vivo bound IgG was found on the intracellular desmosomal plaque and colocalized with DP I as revealed by double labelling with a monoclonal antibody against DP I. This study provides immunomorphological and biochemical evidence of autoantibodies against DP I, a major protein of the cytoplasmic desmosomal plaque in severe mucocutaneous EM. Further studies are required to clarify wether these autoantibodies contribute to the pathogenetic events operative in EM.

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IgG SUBCLASSES SWITCH IN THE PRODUCTION OF ANTI-CADHERIN ANTIBODY IN PEMPHIGUS VULGARIS PATIENTS. A. Razzaque Ahmed, Kallash Bhol, and Aloke Mohimen, Department of Dermatology, Boston University, Boston, MA, USA.

In recent studies, we have demonstrated that the majority of Jewish patients with pemphigus vulgaris(PV) carry the extended haplotype[HLA-B38, SC21, DR4, DQw3] or portions of it (PNAS, 87:7658, 1990). The majority of non-Jewish patients carry the extended haplotype[HLA-B55, SB45, DR6, DQ1] or portions (PNAS 88:5056, 1991). Using a modified sensitive immunoblot, we demonstrated that the majority of the MHC haploidentical first degree relatives produced low levels of the anti-cadherin antibody with a LOD score of 9.82 and a recombination fraction of 1.5 (J. Exp. Med. 177:419, 1993). Using the same assay, we studied the sera of 23 patients with active disease, 20 antibody positive relatives and 15 patients in prolonged (>3 years) remission, to determine the subclasses of IgG. In all the relatives and in the sera of patients in remission, the anti-cadherin antibody was of the IgG_1 subclass. No other subclasses were seen. Of the 23 patients, 17 had both IgG_1 and IgG_4. Of the remaining six, three who lacked IgG_1 had IgG_2 and IgG_3 and three who lacked IgG_4 had only IgG_2. The titer of the IgG_4 anti-cadherin antibody was usually 3-4 dilutions higher than the titer of the IgG_1 autoantibody. These data indicate that at the onset of clinical pemphigus, there is a subclass switch from IgG_1 to IgG_4 with a concomitant significant rise in the titer of the anti-cadherin antibody. Once in prolonged remission there is a reversal to low levels of IgG_1 subclass. These observations highlight the importance of IgG subclass switch in the pathogenesis of PV.

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IDENTIFICATION OF TRANSCRIPTION INITIATION SITE OF THE PEMPHIGUS VULGARIS ANTIGEN GENE AND CELL SPECIFICITY OF EXPRESSION. Stephanie A. Silos, Katsuto Tamai, Kehua Li and Jouni Uitto, Department of Dermatology, Jefferson Medical College, Philadelphia Pa, USA

Pemphigus Vulgaris Antigen (PVA) is a member of the cadherin family and localizes to the desmosomes of keratinocytes. A partial human PVA cDNA has recently been cloned and sequenced (Amagai et al, Cell 67, 869-77, 1991). Using this sequence, we designed several primers which enabled us to localize the transcription initiation site and generate a 468 bp cDNA probe for genomic library screening and Northern analysis. A primer, P3, complementary to -21 to -1 relative to the translation initiation site was radio-labeled, annealed to keratinocyte mRNA and reverse transcribed 68 bp as detected on polyacrylamide gel. Thus, this primer extension study maps the transcription initiation site to approximately position -88. A cDNA probe spanning positions -78 to + 398 was generated using PCR. This probe was used to screen a human lambda Fix II genomic library. Eight positive lambda clones have been isolated and are being characterized to identify the promoter region. This probe was also used to study the expression of PVA mRNA, in keratinocytes under varying Ca^{2+} concentrations and in five different cell types. PVA mRNA was identified only in human keratinocytes, but not in fibroblast, HeLa, KB, or WISH cells. Interestingly, PVA mRNA was detected in keratinocytes grown in the presence of 0.15 mM Ca^{2+} in which both 230-kDa and 180-kDa bullous pemphigoid antigens were also expressed. This finding suggests that PVA expression may not be restricted to differentiated keratinocytes; but is also present in proliferating cells.

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DETECTION OF TYPE VII COLLAGEN GENE MUTATIONS IN DYSTROPHIC FORMS OF EPIDERMOLYSIS BULLOSA PATIENTS BY PCR-SSCP AND PCR-RFLP ANALYSES. Yasushi Suga*,Kazumi Ishidoh**,Shinji Morioka*, Kenji Takamori*,Eiki Kominami** and Hideoki Ogawa*, Department of Dermatology* and Department of Biochemistory**, Juntendo University, School of Medicine, Tokyo, Japan

We performed SSCP (single-strand conformation polymorphism) and RFLP (restriction fragment length polymorphism) analyses to detect mutation of the Type VII collagen gene in dystrophic epidermolysis bullosa (DEB) patients. We designed PCR (polymerase chain reaction) primers for the NC1 domain based on the published sequence and then utilized same for PCR amplification of human genomic DNA from either normal control individuals or DEB affected individuals. SSCP analyses showed that the profiles of single-strand DNAs of DEB patients and those of unaffected individuals were clearly different. RFLP analyses showed that PCR products demonstrated polymorphism with the restriction enzyme Pvu II in DEB families. Subsequent nucleotide sequence analyses suggested that a one point mutation in a Pvu II restriction site caused the aforesaid polymorphism. These above-described analyses clearly detected mutations in the VII collagen gene of DEB patients, suggesting that these techniques are very useful for the detection of various mutations of Type VII collagen when prenatal diagnosis in DEB families is carried out.

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PRENATAL DIAGNOSIS OF JUNCTIONAL EPIDERMOLYSIS BULLOSA BY AMNIOCENTESIS. M. Peter Marinkovich, Guerrino Meneguzzi, Robert E. Burgeson, Jean-Paul Ortonne, Departments of Dermatology at: Oregon Health Sciences University, Portland, OR, USA, Harvard Medical School, Boston, MA, USA, Universtiy of Nice-Sophia Antipolis, Nice, France.

One of the most disabling of the inherited epidermolysis bullosa diseases is the Herlitz junctional form (HJEB) which usually results in death during infancy. We have previously shown that the anchoring filament protein alternatively known as kalinin or nicein is abnormally expressed in tissues of individuals with this disease. We have also shown that antibodies against this protein cause deepidermalization of sections of human skin in situ, suggesting that this protein facilitates dermal-epidermal cohesion. We demonstrate here that this molecule is present in normal second trimester amniotic fluid. The predominant form of kalinin/nicien in amniotic fluid is a partially processed molecule containing subunits of 165 kd, 155 kd, and 140 kd. This molecule purifed from amniotic fluid appears to be biologically active in that it facilitates the attachment of cultured human keratinocytes. Monoclonal antibody K140 recognizes the 140 kd subunit of kalinin/nicein by western blotting of amniotic fluid from all of the 45 normal second trimester control samples, whereas this antibody is unreactive on western blots using second trimester amniotic fluid from 4 pregnancies with HJEB fetuses. Diagnosis of HJEB in these cases was confirmed by absent GB3 reactivity and characteristic electron microscopic features from skin biopsies. These results provide further insight into the pathophysiology of this disease and demonstrate that immunoblotting of amniotic fluid collected by routine second trimester amniocentesis can be used to ascertain the prenatal dignosis of HJEB.

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IDENTIFICATION OF MUTATIONAL HOT SPOTS IN THE SUPRABASAL KERATIN GENES FROM PATIENTS WITH EPIDERMOLYTIC HYPERKERATOSIS. D. Roop, J. Rothnagel, A. Dominey, M. Fisher, S. Axtell, M. Pittelkow, D. Hohl, Mayo Clinic, Rochester, MN; CHUV, Lausanne, Switzerland; Baylor College of Medicine, Houston, TX

Epidermolytic hyperkeratosis (EHK), (bullous congenital ichthyosiform erythroderma), is an autosomal dominant human skin disorder characterized by hystrix-like, ichthyotic, rippled hyperkeratosis that is particularly prominent around joints and folds. Estimates of disease incidence vary from 1 in 100,000 to 1 in 300,000. Ultrastructural studies have implicated the differentiation-specific keratins, K1 and K10 as source of the defect in this disease. Moreover, several studies have linked the disease to the keratin loci on chromosomes 12q and 17q. Recently, we and others have described mutations in highly conserved regions of K1 and K10 in patients with this disease. Structure-function models predict that these mutations would impair normal filament assembly and function. We have extended our earlier studies to included 17 probands of EHK. Sequence analysis of the K1 and K10 gene from these patients have revealed a mutational hot spot within the 1A helix initiating motif of K10. These involve Arginine to Histidine and Arginine to Cysteine substitutions at position 10 of the rod domain. Interestingly, these same substitutions were observed in K14, the basal cell homolog of K10, in patients with epidermolysis bullosa simplex. The large number of mutations found at this position in both keratins K10 and K14 suggests that other epithelia cell disorders will be found with these mutations in their Type I keratins. A good candidate for a gene with a similar defect giving rise to a keratinocyte disorder, is K9 in the skin disease, epidermolytic palmoplanter keratoderma.

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KGF INDUCES TGFα EXPRESSION AND ACTIVATES THE EGF RECEPTOR SIGNALING PATHWAY TO ALTER KERATINOCYTE GROWTH AND DIFFERENTIATION IN VITRO. A.A. Dlugosz, C. Cheng, M.F. Denning, P.J. Dempsey[1], R.J. Coffey, Jr.[1], and S.H. Yuspa, Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, Bethesda, MD 20892, and [1]Departments of Medicine and Cell Biology, Vanderbilt University, Nashville, TN 37232

In addition to being mitogenic for cultured keratinocytes, TGFα and EGF block Ca^{2+}-mediated induction of the spinous cell keratins K1 and K10 while inducing aberrant expression of keratin 8 (K8) (Cheng C. et al., Cell Growth & Differ. *4*: 317-327, 1993). To determine the specificity of this response for EGF receptor (EGFR) ligands, we examined whether members of other cytokine families are capable of inducing the same altered pattern of keratin expression. At a dose mitogenic for mouse primary keratinocytes (10 ng/ml), TGFα, KGF, and aFGF, but not bFGF or IGF-I, block Ca^{2+}-mediated expression of K1 while inducing K8. Since KGF and aFGF (but not bFGF) are ligands for the KGF receptor (KGFR), we explored the possibility that the TGFα/EGFR pathway is an intermediary in signaling through the KGFR. The steady-state level of TGFα mRNA was increased in cells treated with KGF, aFGF, or TGFα, but not bFGF or IGF-I. Similar changes were detected at the protein level: the concentration of secreted TGFα in conditioned medium (CM) from control, KGF-, TGFα-, and aFGF-treated cultures was 54 (±8, S.E.M.), 365 (±50), 146 (±20), and 120 (±50) pg/ml, respectively. A neutralizing TGFα antibody partially blocked the effects of KGF CM on keratin expression and cell growth, suggesting that these responses to KGF are mediated indirectly by secreted TGFα. KGF CM and TGFα both stimulate tyrosine phosphorylation of the EGFR during a 5 minute treatment, and KGF and TGFα both induce EGFR down-regulation after a 24 hour exposure, based on western blot analysis. In addition, binding of ^{125}I-EGF is reduced in keratinocytes treated with either KGF or TGFα at both 8 and 26 hours. Combined, these data indicate that KGF induces TGFα expression in cultured keratinocytes resulting in activation of the EGFR signaling pathway. Our findings strongly suggest that TGFα is the proximal effector of KGF action for at least certain aspects of epidermal growth and differentiation.

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ENDOGENOUS ACTIVATION OF LATENT TGF-β BY INDUCTION OF DIFFERENTIATION OF HUMAN KERATINOCYTES, Yoshihiko Matsui, Koji Hashimoto, Hideo Asada and Kunihiko Yoshikawa, Department of Dermatology, Osaka University School of Medicine, Osaka, Japan

TGF-β is a potent negative growth regulator for human keratinocytes (HK). Endogenous activation of TGF-β is the most important step in vivo. However, its mechanism has not been well characterized in HK. In this study, we studied an involvement of endogenous activation of latent TGF-β in growth inhibition following induction of differentiation. HK were cultured in serum-free MCDB 153 medium containing 0.1 mM Ca^{++} (low Ca^{++}). Differentiation was induced by addition of Ca^{++} to 1.8 mM (Ca^{++} switch). The cell number decreased to 26.8% after Ca^{++} switch. However, addition of anti-TGF-β1 and -β2 antibodies increased cell growth by 66.7%. Since urokinase plasminogen activator (uPA)/plasmin has been reported to be involved in TGF-β activation in endothelial cells, we examined the effect of aprotinin, anti-uPA antibody and anti-plasmin antibody. Addition of these reagents enhanced HK growth to 150.1%, 140.1% and 160.0%. Furthermore, anti-uPA receptor blocking antibody increased cell growth to 146.7%. Even if anti-TGF-β1 and -β2 antibodies were added with these reagents together, additive effect on growth enhancement was not observed. These results indicate that uPA/Plasmin and uPA binding to its receptor are essential for endogenous activation of latent TGF-β induced by differentiation of HK.

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AGE-ASSOCIATED DECREASE IN EPIDERMAL GROWTH FACTOR RECEPTOR FUNCTION.
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Epidermal growth factor (EGF) is an important fibroblast mitogen whose effectiveness decreases with aging, although extensive studies in the Hayflick model of in vitro senescence have failed to reveal a mechanism. Using early passage human fibroblasts derived from newborn (NB), young adult (YA), and old adult (OA) donors we show an age-associated loss of proliferative response to EGF that parallels the number of EGF receptors (EGFR) per cell: NB 275,000 ± 6951; YA 135,000 ± 4887 and OA 50,000 ± 4913 EGFR/cell (as determined by ^{125}I-EGF binding). These differences ($p<.001$) were confirmed by FACS analysis using monoclonal antibodies. A population of high affinity receptors was revealed by Scatchard analysis to be present on NB but absent on YA and OA cells. Maximal internalization of EGF-receptor complexes occurred in NB within 60 min as compared to 90 min in YA and OA. The effect of age on the EGF/EGFR endocytic pathway was examined by Percoll gradient centrifugation and sequential enzyme marker localization analysis as well as by confocal microscopy. EGF/EGFR complexes remained associated with the plasma membrane twice as long in YA as in NB cells and 5 times as long in OA as in NB cells. Progression of the complexes through the endosomal to the lysosomal compartment was also prolonged with age. Confocal analysis utilizing EGF-Rhodamine confirmed the slower progression of EGF through the endocytic compartments with increasing age. After EGF stimulation total EGFR protein levels fluctuated in NB, decreasing to 25% of baseline levels at 2hr and 8hr, reflecting cycles of receptor degradation and synthesis. Adult fibroblasts did not show modulations and baseline EGFR protein was only 1/3rd that of NB and northern analysis showed a decreased EGFR mRNA in OA as compared to NB. Moreover NB phosphorylated EGFR within 2 min after EGF stimulation while OA phosphorylated EGFR 15 min after EGF stimulation. The striking age-associated differences in EGF binding and processing in early passage cells were not detectable after 10-15 population doublings, explaining the apparent discrepancy with the Hayflick model. Our findings reveal major age-associated impairment in the EGF/EGFR signal transduction pathway. These impairments may contribute to slow wound healing in the elderly.

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UVB IRRADIATION INCREASES ENDOTHELIN-1 AND TYPE B ENDOTHELIN RECEPTOR EXPRESSIONS IN CULTURED HUMAN KERATINOCYTES.
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Endothelin-1 (ET-1) is a potent vasoconstrictor peptide. Recently it has been demonstrated that ET-1 acts as an autocrine/paracrine growth factor in normal human keratinocytes. In this study, the effect of UVB irradiation on ET-1 and ET receptor expressions was examined using normal human keratinocytes cultured in modified MCDB 153 medium. ET-1-like immunoreactivity was secreted in conditioned medium at the level of 2.4 pg/day/10^6 cells, and UVB irradiation up to 10 mJ/cm^2 increased ET-1 secretion dose dependently. The addition of neutralizing antibodies against IL-1α and TNF-α suppressed the UVB-induced ET-1 secretion to non-irradiated cell level. ET-1 mRNA detected by RT-PCR and type B ET receptor mRNA detected by northern blotting were stimulated by UVB irradiation. Antibodies against IL-1α and TNF-α reduced type B ET receptor expression of UVB-irradiated cells. These results suggest that ET-1 and ET B receptor expressions in keratinocytes are modulated by UVB irradiation through IL-1α and TNF-α.

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PHOSPHOLIPASE C - MEDIATED SIGNALTRANSDUCTION IS REDUCED DURING THE COURSE OF KERATINOCYTE DIFFERENTIATION.
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Phospholipase C (PLC) - mediated release of inositol(1,4,5) trisphosphate (Ins(1,4,5)P_3) and diacylglycerol plays a fundamental role in signal transduction and contributes to the regulation of cellular functions, e.g. proliferation. We recently described a decrease of bradykinin - induced [^{3}H]Ins(1,4,5)P_3 - generation during the logarythmic growth phase and differentiation of HaCaT keratinocytes which was studied in more detail in the present investigation. This effect is not related to a potential down - regulation of the bradykinin receptor, since direct activation of PLC by A23187 showed identical results. Expression of Gq_α, the G protein subunit which activates PLC, showed no differences between proliferating and differentiated HaCaT cells. Analysis of the PLC - substrates phosphatidylinositol (PI), PIP, and PIP_2 by thin layer chromatography revealed a steady decrease in the amount of these membrane phospholipids which paralleled exactly the time - course of reduced Ins(1,4,5)P_3 formation. Expression of protein kinase C (PKC) isoenzymes PKCα, PKCß, and PKCε was enhanced in differentiated keratinocytes as determined by immunoblots. Since PKC represents a negative feedback signal, the enhanced expression might further contribute to the observed down-regulation of Ins(1,4,5)P_3. The reduced Ins(1,4,5)P_3 formation suggests not only an altered reactivity of differentiated keratinocytes to external mediators but might also contribute to the regulation of keratinocyte proliferation and differentiation.

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SIGNAL TRANSDUCTIONS AND GENE REGULATIONS BY CUTANEOUS NEUROPEPTIDES THROUGH ACTIVATION OF PROTEIN KINASE A AND C IN CULTURED KERATINOCYTES, MELANOCYTES, FIBROBLASTS.
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Peptides released from peripheral cutaneous neurons are known to be involved in various skin diseases as immunomodulators. To examine the direct effects of cutaneous neuropeptides on the human skin, intracellular signals (IPs, Ca influx and cAMP) and gene expressions following to the activation of protein kinase A and C were studied. We found that bradykinin stimulated PKC in keratinocytes and fibroblasts through the inositol phosphate turnover and Ca influx, and that endothelin activated PKC in fibroblasts and melanocytes. The rapid transcriptional activation of a group of early response genes including c-*fos* and *egr*-1 genes was clearly observed following to C kinase activation by bradykinin and endothelin in keratinocytes and melanocytes, respectively. The expressions of those genes were eliminated by the addition of the inhibitors to PKC. On the other hand, peptides which stimulated PKA through cAMP formation did not enhance the expression of those genes including CGRP, VIP and PHM in keratinocytes, and MSH and CGRP in melanocytes and fibroblasts. Furthermore we found that the inhibitory effect of neuropeptide Y, opioid and somatostatin on cAMP formation in keratinocytes, melanocytes and fibroblasts, respectively.

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PRENYLATED PROTEINS ARE INVOLVED IN RESPONSES TO INFLAMMATORY AND PROLIFERATIVE STIMULI IN HUMAN KERATINOCYTES.
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Proteins with covalently attached isoprenoid groups derived from mevalonate have been implicated in signaling pathways. We previously showed receptor-mediated inositol 1,4,5-trisphosphate (IP_3) production to be down-regulated by depleting keratinocytes of isoprenoids. We have now investigated protein prenylation and the effects of direct inhibitors in the Hacat human keratinocyte line. Protein farnesyl and geranylgeranyl transferases (PFT, PGT) were assayed using synthetic peptide substrates. In cells, IP_3 was measured by a specific binding protein assay, or [^{3}H]IP production was measured after pre-loading with [^{3}H]inositol. Hacat cells incorporated [^{3}H]mevalonate into proteins of 8 kD, 20-30 kD and 50 kD, seen by gel electrophoresis and autoradiography. PFT and PGT enzymes were isolated from cytosol of Hacat cells and shown to be inhibited by zaragozic acids B (ZAB) and C. The inflammatory mediator bradykinin (BK) caused a rapid (15 sec) increase in IP_3 mass, while epidermal growth factor (EGF) caused only a slow (10 min) accumulation of [^{3}H]IPs. Treatment of cells with ZAB (0.1 to 10 μM) resulted in a dose-dependent, up to 50% reduction in the IP_3 response to BK. In contrast, inhibition of cholesterol biosynthesis by the specific squalene epoxidase inhibitor NB-598 had no effect. EGF-stimulated [^{3}H]IP production was also inhibited by blocking prenylation. We conclude that keratinocytes produce prenylated proteins which play an important role in cellular signaling responses to both inflammatory and proliferative stimuli.

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EFFECT OF BOTULINUM C3 EXOENZYME ON THE CELL GROWTH AND CYTOSKELETAL ORGANIZATION OF TRANSFORMED HUMAN EPIDERMAL CELLS IN CULTURE; A POSSIBLE ROLE FOR *rho* PROTEIN.
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Botulinum ADP-ribosyltransferase C3 exoenzyme specifically ADP-ribosylates *rho* gene products, a group of small molecular weight GTP-binding and *ras* related proteins. The ADP-ribosylation reaction occurs at a putative effector domain of *rho* proteins, interferes with the signal transduction pathway and blocks stimulus-evoked cell adhesion in human platelets and lymphocytes. Previously, we reported that ADP-ribosylation of *rho* proteins by C3 exoenzyme delays cell cycle transition at the G_1/S phase in Swiss 3T3 cells (Yamamoto *et al.*: Oncogene 8; in press, 1993). To clarify the role of the *rho* gene products in epidermal cells, we studied the effect of C3 exoenzyme on cell growth and cytoskeletal organization in the transformed human epidermal cell line HSC-1 Addition of C3 exoenzyme to HCS-1 cells reduced the cell growth rate; 72-hr-treatment with C3 exoenzyme at 5, 10, 30 and 60 μg/ml culture medium resulted in 22, 28, 61 and 68% growth inhibition compared to untreated control, respectively. Morphologically, the HSC-1 cells became more rounded with beaded processes in a time- and dose-dependent manner. Immnofluorescence revealed that under this condition, actin stress fibers were disassembled, whereas keratin intermediate filaments were not affected. These results suggest that *rho* proteins are involved in cell growth and at least partially regulate the assembly of actin stress fibers in transformed epidermal cells.

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VEHICLE EFFECTS ON INTERLEUKIN 1ß ANTISENSE METHYLPHOSPHONATE OLIGONUCLEOTIDE DELIVERY IN VITRO

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Antisense therapy is a rapidly expanding field whereby cells can be provided the ability to inhibit the synthesis of a specific protein. Since antisense oligomers react with a specific sequence of mRNA, dose and toxicity should be minimal. Topical formulations of antisense oligomers provide the benefit of being non-invasive and avoiding first-pass metabolism (versus oral dosing). To test the feasibility of delivering antisense topically, six formulations and an internal standard all containing ^{3}H-labeled human interleukin-1ß antisense methylphosphonate oligonucleotide (as-IL-1ß, 13-mer) were tested in vitro to determine antisense oligomer flux through and tissue distribution in human abdominal skin. Franz cell receptor phase concentrations were monitored for 48 hours. After 48 hours, the stratum corneum, epidermis, and dermis were individually analyzed for ^{3}H content. The internal standard vehicle (water/acetonitrile 50% v/v) provided the greatest flux of as-IL-1ß through the skin. The tested formulations provided less flux, but some had greater levels in the stratum corneum, epidermis, and dermis than the internal standard. Distribution studies reveal that radiolabel is most concentrated in the stratum corneum regardless of vehicle. Epidermis and dermis concentrations of as-IL-1ß were essentially equivalent and 10 to 20 times less than stratum corneum concentrations with one exception. Therefore, the tested formulations do influence the distribution of as-IL-1ß in the skin.

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NITRIC OXIDE SYNTHASE; IMMUNOLOCALISATION AND FUNCTION IN NORMAL HUMAN SKIN.

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Cutaneous vascular tone is mediated by both neuronal and endothelial mechanisms. In large blood vessels the constitutive release of nitric oxide (NO) by the action of NO synthase on L- arginine is important in endothelium-dependent vasodilatation. We have investigated the presence of NO synthase in human skin by immunohistochemistry and its action by studying the effects of a competitive inhibitor of NO synthase, N^G-monomethyl-L-arginine (L-NMMA) and its inactive isomer N^G-monomethyl-D-arginine (D-NMMA) on cutaneous blood flow at rest and after local warming.

Digital cutaneous biopsies from healthy volunteers (n=6) were immunostained with antisera to constitutive and inducible NO Synthase and its endothelial and neuronal isoforms. In healthy volunteers (n=6) 25 µl of L-NMMA 1mmol, D-NMMA 1mmol and normal saline were injected intradermally (ID) into each forearm. One forearm, 1.5 minutes later, was immersed for 30 secs in water at 45^{0}C. Blood flow was measured by laser-Doppler flowmetry (LDF) before injection and at each injection site and also at a site in the locally heated forearm prior to and after warming. Pallor diameter was measured by planimetry and its area calculated. The same forearm was reimmersed for 30 secs prior to repeating the measurements on both forearms at 10, 20 and 40 minutes post injection. In all biopsies there was positive immunostaining only for constitutive endothelial NO synthase in the dermal microvascular endothelium. In the unwarmed forearm ID L-NMMA and D-NMMA did not produce pallor or change in blood flow. In the warmed forearm L-NMMA produced pallor at the injection site at 20 and 40 minutes and significantly reduced blood flow ($p<0.05$ Wilcoxen signed rank) compared to D-NMMA and to the heat-induced erythema ($p< 0.05$). D-NMMA and saline did not produce pallor or a significant reduction in blood flow.

These results indicate that NO synthase is present in the cutaneous microvascular endothelium and that intradermal injection of L-NMMA inhibits vasodilation due to local warming by competitive inhibition of NO synthase which thus may be important in maintaining blood flow in the skin.

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RETINOL ELICITS EPIDERMAL ACANTHOSIS AND CRABP-II mRNA EXPRESSION, BUT INSIGNIFICANT ERYTHEMA WHEN TOPICALLY APPLIED TO HUMAN SKIN.

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All-trans retinoic acid (RA) and *all-trans* retinol (ROL) are important regulators of epidermal growth and differentiation. Topically applied to human skin, RA consistently produces erythema, epidermal acanthosis, spongiosis and cellular retinoic acid binding protein (CRABP) II mRNA expression. Biologic effects of topical ROL in human skin have not been fully characterized. We investigated the clinical, histological, and molecular responses of normal human skin to topical ROL and compared them to those of topical RA. Metabolites derived from ROL application were also measured and compared to those from the vehicle-treated skin. 100 µl/18 cm^2 of vehicle (70% ethanol/30% propylene glycol), 0.4%, 0.8% or 1.6% ROL or 0.025% RA were applied to normal buttock skin in a double-blind fashion and occluded for 4 days. RA produced a significant 3.7 fold increase in erythema score as compared to vehicle ($n = 10$, $P < 0.01$), whereas ROL produced trace erythema which was clinically and statistically insignificant. However, in the face of no/trace erythema, ROL induced significant increases in epidermal acanthosis (1.5 fold at 1.6%, $P < 0.01$) and spongiosis (4.1 fold at 1.6%, $P < 0.01$), approaching the level of 0.025% RA relative to vehicle treatment ($n = 10$) [(acanthosis) 1.6 fold, $P < 0.01$; (spongiosis) 4.1 fold, $P < 0.01$], when scored blindly. All ROL (0.4%, 0.8% and 1.6%) induced CRABP-II mRNA to a level 3-6 fold over vehicle reaching the level of induction by 0.025% RA. Metabolism of ROL in epidermis determined by reverse phase HPLC revealed increased content of 13-cis ROL and retinyl esters ($n = 21$). ROL content in experimental solutions remained stable above 99% purity level during the study. RA was detected in only 2 of 21 ROL treated samples at trace levels (detection limit 1 ng). These data demonstrate that topical ROL induces: 1) classical RA-type epidermal histology and CRABP-II mRNA expression; 2) no detectable increase in RA formation; and 3) insignificant erythema. If this 4 day ROL exposure predicts long term response (as is true for RA), then topical ROL has the potential to deliver clinical changes seen with RA therapy but with a markedly improved clinical tolerance (ie, reduced irritation).

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SUCCESSFUL PROTECTION OF ANTIGEN SPECIFIC DERMATITIC REACTION IN ATOPIC DERMATITIS BY TOPICAL O-ACYLCERAMIDES.

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Since the impaired protective function of the stratum corneum due to loss of ceramides in atopic dermatitis (AD), we hypothesized the exacerbation of antigen penetration to cutaneous immune system in AD. To confirm this hypothesis, we evaluated the protective effect of topical O-acylceramides (OAC) in patch testing with dermatophagoides antigen. Pretreatment with 5 % OAC cream successfully prevented the eczematous reactions of the patch test sites of 18 in 24 AD patients, but 7 in 24 AD by placebo. Histopathological study of the patch tested sites revealed that topical OAC significantly suppressed the dermal eosinophil cationic protein, major basic protein and dermatophagoides antigen deposition compared with the placebo treated site. Four weeks clinical application of this OAC cream improved all of 14 AD patients, and 4 AD patients became free from topical corticosteroid. This study confirmed the importance of OAC and barrier function in AD.

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A STRONG ASSOCIATION OF PSORIATIC ARTHRITIS WITH A SPECIFIC HLA HAPLOTYPE CARRYING A2-B46-DR8-DQ6.

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Psoriatic arthritis (PA) is an inflammatory disease under genetic and environmental factors. A strong association between HLA-B27 and PA has been noted both in Caucasians and the Japanese.

We report here further evidence for a strong association between HLA and PA susceptibility.

HLA class I and II antigens were analyzed in 19 Japanese patients with PA, using the criteria proposed by Moll and Wright. Serological HLA association study showed that PA had significant associations with HLA-A2 ($p < 0.01$) and B46 ($p < 0.05$), in addition to B27 ($p < 0.01$). The frequencies of HLA-Cw6, Cw7, DR8 and DQ6 in PA were also increased, but without any statistical significance. In the Japanese population, HLA-A2-B46-DR8(DRB1*0803)-DQ6(DQA1*0103/DQB1*0601) haplotype is known to be one of the common HLA haplotypes. The three-locus haplotype frequency (Hf) of HLA-A2-B46-DR8 was very high (Hf = 0.195) as compared with 0.006 of normal controls. The relative risk of developing PA was highest at 9.21 in the HLA-A2 positive individuals. The associations between HLA-B46, DR8 or DQ6 and PA were considered to be secondary because of the strong linkage disequilibrium between HLA-A2 and B46, DR8 or DQ6. The HLA-A2-B46-DR8-DQ6 haplotype-positive PA patients seem to show high immune responses in vivo to a streptococcal cell wall antigen.

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EVIDENCE FOR AN HTLV-I LIKE INFECTION IN HTLV-I SERONEGATIVE CUTANEOUS LYMPHOMAS PRESENTING AS HYPEREOSINOPHILIC SYNDROME.

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We report the identification of an HTLV-I related retrovirus in three cases of HTLV-I seronegative cutaneous T-cell lymphomas with hypereosinophilia. Two cases had very atypical cutaneous lesions, and presented for several years as idiopathic hypereosinophilic syndrome before the diagnosis of cutaneous lymphoma could be established. The third patient presented as a mycosis fungoides with hypereosinophilia. Only two originated from endemic areas for HTLV-I infection. Patients had marked eosinophilia, and increased levels of IgE and soluble IL2 receptor. Cutaneous biopsies showed a pleomorphic lymphoma, small and medium-sized cells. The phenotype of atypical lymphocytes was CD3+ CD2+ CD5+ CD4+ CD8- CD7-. Patients were HIV-1, HIV-2, and HTLV-I seronegative in ELISA and Western Blot. No HTLV sequence was detected in uncultured peripheral blood mononuclear cells (PBMC). PBMC were cultured with PHA and IL2, alone or with umbilical cord blood cells. After 4 weeks, a positive HTLV intracytoplasmic fluorescence was observed.Three immortalized IL2-independent T-cell lines were obtained. Their phenotype was CD2+ CD3+ CD4+ CD25+. A radioimmunoprecipitation assay demonstrated the presence of HTLV-I p19 and p24 gag proteins and gp61 env protein. The electron microscopy of the cell lines showed few typical type C particles. PCR amplification technique detected HTLV-I genomic sequences in gag, pol and tax regions, using HTLV-I specific oligonucleotides primers and probes. Restriction analysis showed a very close relationship with HTLV-I. Complete sequencing is being performed. These cases suggest that a variant of HTLV-I or an unknown retrovirus sharing sequence homologies with HTLV-I may be the causative agent of some cases of cutaneous T cell lymphomas with eosinophilia.

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CYCLOSPORINE A, PSC 833 AND FK 506, BUT NOT CYCLOSPORINE H AND RAPAMYCIN, INDUCE ANAGEN AND INHIBIT CATAGEN IN MURINE SKIN.

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The ability to manipulate hair cycling in man so that hair growth (anagen) is induced and follicle regression (catagen) is prevented, would signify a breakthrough in the treatment of hair loss. Therefore, the development of models for testing the anagen-inducing and catagen-inhibitory properties of candidate drugs and the definition of key target molecules for hair growth-modulatory drugs are urgently required. Here, we show that the C 57 BL-6 mouse is a model well-suited to serve both purposes and that anagen induction and catagen inhibition are achievable in vivo. Telogen mice, or mice in depilation-induced anagen, were injected 3 x i.p. with the immunosuppressive immunophilin (IP) ligands cyclosporine A (CsA), FK 506 or rapamycin (RPM, no calcineurin-interaction), with the non-immunosuppressive, non-IP-binding Cs analogs CsH or PSC 833. By assessing the corresponding changes in skin color, it was judged macroscopically and verified by histology whether anagen was induced or catagen inhibited compared to vehicle controls (olive oil + ethanol). Comparatively high doses of CsA and FK 506, but not of CsH or RPM, dose-dependently and differentially induced anagen and retarded catagen development in mice. When PSC 833 was injected, similar, yet weaker hair growth effects as with CsA and FK 506 were noted. This is the first report of any hair growth-modulatory effects of FK 506 and introduces the IPL CsA and FK 506 as potent catagen blockers. Interaction with the calcium/calmodulin-dependent phosphatase calcineurin, not IP-binding, may be the key step for manipulating hair cycling, while the PSC 833 effects suggest that other signal transduction pathways are also involved. The dissection and selective targeting of the molecular pathway(s) responsible for the hair growth effects of CsA, FK 506, and PSC 833 should help to develop effective, less toxic drugs for the therapeutic manipulation of hair cycling.

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LOCALIZATION OF GLUCOCEREBROSIDASE WITHIN MURINE STRATUM CORNEUM.

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The intercellular lipids of stratum corneum, enriched in ceramides, are critical for the mammalian permeability barrier. Recent studies have shown that the conversion of epidermal glucosylceramides (GlcCer) to ceramides (Cer) by β-Glucocerebrosidase(GCase) is important both for permeability barrier function and for lamellar bilayer formation in the intercellular domains of the SC (Holleran et al, J.Clin.Invest. 91:1656,1993). To investigate whether the conversion of GlcCer to Cer occurs in SC, we have localized GCase activity within murine epidermis and SC, characterized this SC GCase activity, and compared the activity of GCase to other hydrolases in epidermis and SC. Using three methods for preparation of isolated SC, including *trypsinization, tape-stripping, and cyanoacrylate glue-stripping*, we have observed significant GCase activity within whole SC, which persists into the outer SC layers. Characterization of the SC GCase showed activity to be stimulated > 10-fold by 5 mM sodium taurocholate (pH 5.2), and completely inhibited by a specific inhibitor of GCase, bromoconduritol B-epoxide (99.6±0.6% at 0.1 mM). Moreover, SC activity represented approximately 25% of total epidermal GCase activity when expressed per surface area (per cm^2), a higher percentage than any other hydrolases measured. Using isolated membrane couplets, approximately 20% of SC GCase activity was further localized to membrane domains. These findings demonstrate that GC to Cer conversion within the outer epidermis can be attributed to GCase *activity within the extracellular spaces in the stratum corneum.*

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ROLE OF MAC-1 IN NEUTROPHIL ATTACHMENT TO THE SERUM-TREATED STRATUM CORNEUM.

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Neutrophil accumulation in the stratum corneum (SC) is a characteristic dermatopathological finding in various aseptical pustular dermatoses. In order to elucidate the pathomechanism of such kind of phenomenon, we examined whether neutrophils attach to SC *in vitro* and further investigated the mechanism of neutrophil binding to the SC.

SC sheets were prepared on sterile slide glasses by using double adhesive tape. After blocking non-specific binding of human neutrophils to SC by PBS with 1%BSA (medium), these SC sheets were incubated with human non-treated or heat-inactivated (56°C, 30min) serum at 37°C for 45min. After SC sheets were washed thoroughly with *medium, 50μl of 1x10⁶ cells/ml of neutrophil suspension in medium was applied on them* and incubated at 37°C for 45min. After removing non-attached cells, attached cells were stained with Giemsa and counted with a computed image analyzer. In five different experiments, significant numbers of neutrophils attached to serum-treated SC (55 ± 3 cells/field), while heat inactivation of serum caused the marked reduction of attached cell numbers (5 ± 1 cells/field), suggesting that this phenomenon is mediated by complement activation. We also used factor B-depleted serum (BDS) or C1q-depleted serum (CDS) to characterize this attachment. Although CDS did not mitigate this neutrophil binding significantly (85% of control), use of BDS resulted in significant decrease (13% of control) in attached cell numbers, indicating that neutrophils attach to SC via the alternative pathway activation. Finally, in order to examine what kind of ligand-receptor interaction mediates this notable phenomenon, we added anti-CR1, anti-LFA-1, or anti-Mac-1 (CR3) antibody to this system. Neither anti-CR1 or anti-LFA-1 antibody inhibited this neutrophil adhesion, while anti-Mac-1 antibody blocked more than 80% of neutrophil binding. This suggests that this neutrophil attachment was mediated by binding of Mac-1 *on neutrophils to C3bi on SC surfaces.*

In conclusion, our present study demonstrates that neutrophils attach to serum-treated SC through an interaction of Mac-1 with C3bi on the SC.

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VCAM-1 EXPRESSION IN MURINE CUTANEOUS LEISHMANIASIS AND ANGIOGENESIS.

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Vascular cell adhesion molecule-1 (VCAM-1) mediates adhesion of mononuclear cells to cytokine-activated endothelial cells via binding of the integrin very late antigen-4 (VLA-4); this study was initiated to investigate its role in the onset and the course of two different models of murine experimental inflammation: cutaneous leishmaniasis and angiogenesis. Cryostat sections at various time intervals after induction of leishmaniasis (footpad, infected with Leishmania major promastigotes) and angiogenesis (cornea, cauterized with an applicator stick of silver nitrate) were analysed immunohistochemically using the mAb M/K-1.9 against murine VCAM-1 and mAbs against various subtypes of lymphocytes and monocytes/macrophages. The extent of the inflammatory response was investigated by measuring footpad thickness and evaluating the degree of neovascularization. During the course of both leishmaniasis and angiogenesis VCAM-1 was found to be increasingly expressed by vascular endothelium of pre-existing and new vessels. In leishmaniasis the intensity of the VCAM-1 expression correlated with the number of both infiltrating lymphocytes and macrophages. During corneal neovascularization the degree of VCAM-1 expression paralled the amount of infiltrating mature macrophages, but not lymphocytes. In both models no correlation, however, existed between VCAM-1 expression and the percentage of infiltrating granulocytes and immature macrophages. We additionally analyzed by flow cytometry regulation of VCAM-1 expression on two murine endothelial cell lines. As in the human system VCAM-1 was found to be strongly up-regulated by TNF-α and, to a lesser extent, by IL-1 and LPS, whereas IFN-γ revealed no effect. Our study suggests i. a correlation between VCAM-1 expression and the extent of the inflammatory response and as well as the number and type of the infiltrating cells and ii. comparable regulation of VCAM-1 expression in human and murine system. Thus, investigation of murine experimental models of inflammation allows insight in regulation and function of VCAM-1 in human diseases.

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MULTIPLE MECHANISMS MEDIATE ADHERENCE OF *CANDIDA ALBICANS* TO CULTURED HUMAN KERATINOCYTES.

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The opportunistic yeast *C. albicans* is the most frequent causative agent of cutaneous candidosis. Adherence of *C. albicans* to cells in various target tissues is regarded as an important first step in the pathogenesis of candidosis. To gain a better understanding of the molecular mechanisms involved in *C. albicans* adherence to keratinocytes (KC), we established an adherence model that uses cultured human KC derived from neonatal foreskin as target cells. It is evident from the performed experiments that *C. albicans* adherence to human KC is a complex process of multiple molecular mechanisms. Attachment of *C. albicans* increased with increasing inocula of yeast cells by coaggregational mechanisms and with higher incubation temperatures leading to optimum results at 37°C. All of the *C. albicans* strains tested showed very high attachment in contrast to less pathogenic *Candida* spp. of which *C. tropicalis* was the best adherent species. Pepstatin A, an inhibitor of the *C. albicans* secretory protease was very effective in inhibiting adherence at concentrations as low as 1 μM (45% inhibition). The metabolic inhibitor Na-azide very potently reduced *C. albicans* adherence most probably by down-regulation of KC receptors (>50% inhibition at 1 mM). As opposed to *C. albicans* adherence to endothelial cells, only partial inhibitory effects of fibronectin or RGD-containing peptides in *C. albicans* adherence to KC were noted. However, an adherence related peptide derived from the laminin B-chain *(containing the sequence YIGSR) inhibited C. albicans* adherence to human KC by *more than 75%. Another dose-dependent inhibitor of C. albicans adherence is the connective tissue* protein collagen type III. Of the various sugar molecules tested, the most potent inhibitor of *C. albicans* adherence was the amino sugar glucosamine. Collectively, these data allow the following conclusions: **(i)** *C. albicans* adherence to cultured human KC is mediated by multiple mechanisms such as protein-protein interactions (e.g. YIGSR-mediated), lectin-carbohydrate interactions, secretion of *C. albicans* secretory protease, and yeast coaggregation. **(ii)** Adherence of *C. albicans* to human KC, in contrast to endothelial cells, cannot be sufficiently inhibited by RGD peptides.

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DIFFERENTIAL EFFECTS OF ULTRAVIOLET B TREATMENT ON THE ADHESIVE PROPERTIES AND LIGAND EXPRESSION OF PSORIATIC DERMAL MICROVASCULAR ENDOTHELIAL CELLS.

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Psoriasis is characterized by intense CD4 T lymphocytic infiltrates. Our previous studies have shown that psoriatic dermal microvascular endothelial cells (DMEC), unlike normal DMEC, express endothelial ligands such as ICAM-1, ELAM-1 and VCAM-1 and promote the specific adherence of memory T cell subset. Ultraviolet B (UVB) irradiation is a major treatment modality for psoriasis, although the mechanism is unclear. In the present study, we compared the adhesive properties of DMEC in untreated and UVB-treated psoriatic plaques and correlated lymphocyte adhesion to expression of ICAM-1, ELAM-1 and VCAM-1. For this purpose, five patients with large plaque type of psoriasis received UVB light treatment twice a day with an initial dose of 20 mJ/cm^2, increased by 5 mJ/cm^2 daily. Peripheral blood and 4 mm punch biopsies were obtained before and during different periods of treatment. Control patients were treated with emollient alone. The results showed that treatment of psoriatic plaques with UVB by day 6 decreased the adhesiveness of psoriatic DMEC for human T cells by >80% as compared to plaques treated with emollient alone. The decrease in binding was associated with reduction in the expression of ICAM-1 and ELAM-1 on psoriatic DMEC. In contrast, the expression of VCAM-1 on psoriatic DMEC was up-regulated following UVB treatment, implying that lymphocyte binding is not promoted by this ligand. Interestingly, psoriatic lesions which were covered with occlusive dressings during UVB treatment also demonstrated similar decreases in the adhesion of lymphocytes and ICAM-1 and ELAM-1 expression, suggesting a systemic effect of UVB. Our finding supported the hypothesis that UVB treatment may exert a therapeutic effect at the level of endothelial adhesiveness for lymphocytes.

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SUBSTANCE P AUGMENTS INTERFERON-GAMMA (IFN-γ) BUT NOT TUMOR NECROSIS FACTOR (TNF-α) MEDIATED INDUCTION OF KERATINOCYTE ICAM-1 EXPRESSION. R.S. Mitra and B.J. Nickoloff, Dept. of Path. Univ. of Mich., Ann Arbor, MI.

Early cellular and molecular events in inflamed skin include the active participation of epidermal KC and dermal mast cells which can produce diffusible mediators such as TNF-α and histamine. Rapid induction of KC ICAM-1 is observed following a highly diverse array of stimuli which provoke irritant, inflammatory, allergic and immune reactions. Recently it has been shown that the neurologic system may have a role in some skin diseases and neuropeptides are believed to be involved. Substance P (SP) releases histamine in skin in-vivo and from isolated mast cells in-vitro. The present work is aimed to understand the basic mechanism of skin reactions by studying interactions between soluble mediators of inflammation in cultured KC in the induction of ICAM-1. Multipassaged human KCs grown in a serum free, low calcium medium were exposed to various agents singly or in combination. After 48 hours, cell surface ICAM-1 expression was quantified by indirect immunofluorescence staining and FACS analysis. SP (0.05 mM - 0.5 mM) by itself failed to induce KC ICAM-1 expression (mean channel fluorescence; χ= 10 versus untreated control of 7); however, SP augmented IFN-γ (10 U/ml) induced ICAM-1 in a concentration dependent fashion: χ= values are: 68, 115, 137 and 244 at concentrations of 0, 0.05, 0.1 and 0.5 mM respectively. In contrast SP at these concentrations had no effect on TNF-α (500 U/ml) induced ICAM-1. Thus, it appears that SP by itself can modulate cytokine actions in cultured KC. While histamine had no significant effect on IFN-γ induced ICAM-1, TNF-α (500 U/ml, 48 hrs) - mediated ICAM-1 expression was significantly increased in the presence of 5mM histamine (x values = 3, 26, 8 and 48 for untreated control, TNF-α, histamine, and TNF-α + histamine); L-histidine had no effect. By Scatchard analysis a single class of high affinity TNF receptors was detected in KC (KD = 0.25 ± 0.03 nM, receptor sites/cell 1600 ± 150) which remained unaltered by histamine exposure. We conclude that SP and histamine can contribute to the cytokine network of inflammation/immune reactions and provide evidence for potential cross-talk between epidermal and dermal cell types involving soluble mediators of skin inflammation.

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VCAM-1 IS INCREASINGLY EXPRESSED IN DERMAL NERVE FIBRES DURING MURINE EXPERIMENTAL CONTACT DERMATITIS.

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Vascular cell adhesion molecule-1 (VCAM-1) is known to be induced on cytokine-activated human endothelial cells and to mediate adhesion of mononuclear leukocytes via binding to the integrin VLA-4. Recent studies have shown that VCAM-1 is also expressed on nonvascular cell types among them cultered human neural cells. To further elucidate the role of VCAM-1 *in vivo* we investigated immunohistochemically the expression of VCAM-1 during experimental irritant (ICD) and allergic (ACD) contact dermatitis of BALB/c mice using mAb M/K-1.9 against murine VCAM-1. Cryostat ear sections obtained at various time points after induction of ICD (croton oil) or ACD (DNFB) were analyzed for VCAM-1 expression by light microscopy and confocal laser scanning microscopy using a semiquantitative scoring system. During both ICD and ACD, VCAM-1 was not only increasingly expressed on vascular endothelia but also on axons of nerve fibres. In both types of contact dermatitis neural VCAM-1 expression paralleled the extent of the cellular infiltrate the latter being mainly comprised of macrophages. Additionally, preliminary studies on VCAM-1 expression during ICD and ACD in C57BL/6 mice which are known to respond with smaller cellular infiltrate to inflammatory stimuli compared to BALB/c mice, showed a lower extent of neural and endothelial VCAM-1 expression. We furthermore analyzed immunohistochemically the effect of intradermal injection (footpads of BALB/c mice) of murine rTNF-*α* which is known to induce VCAM-1 on human neural cells *in vitro*. As soon as 6 hours after injection, VCAM-1 was found being strongly up-regulated on axons of nerve fibre bundles as well as on vascular endothelia. Our results document for the first time an induction of VCAM-1 on peripheral nerve fibres *in vivo* during inflammatory reactions. As recently shown for human neural cells *in vitro*, up-regulation of neural VCAM-1 expression *in vivo* may also be caused at least partially by TNF-*α* which is known to be released by infiltrating macrophages during inflammatory processes.

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REGULATION OF ACTIN-POLYMERIZATION IN HUMAN EOSINOPHILS BY CHEMOTACTIC MEDIATORS - "PRIMING"-EFFECT OF IL-5 AND GM-CSF

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Alteration of the cytoskeleton is a prerequisite for the motility and chemotaxis of leukocytes. Eosinophils (EO) are major effector cells in allergic late phase reaction and especially in atopic dermatitis. To obtain more information about EO activation in inflammatory reactions, in the present study regulation of actin-polymerization was investigated in highly purified EO (≥95%) from healthy individuals by flow-cytometry using NBD-phallacidin, which specifically binds to polymerized actin molecules (F-actin). Stimulation of EO by RANTES, PAF and C5a in-vitro dose-dependently induced a significant and reversible polymerization of actin. Polymerization of actin could be blocked by pertussis-toxin, which indicates an involvement of G_i-proteins. In contrast, stimulation of EO by cytokines such as IL-3, IL-5, GM-CSF, TNFα, IL-8 and MCP-1 in physiological concentrations did not significantly influence actin-polymerization. However, after preincubation of EO with IL-5 or GM-CSF, a synergistic induction of actin-polymerization induced by RANTES, PAF and C5a could be observed. In contrast, this synergism was not observed by preincubation with IL-3, TNFα, IL-8 and MCP-1. In summary, the present study for the first time demonstrated, that RANTES, PAF and C5a are potent activators of actin-polymerization in human EO. Moreover, IL-5 and GM-CSF have the capacity of a "priming" of EO in respect of actin-polymerization. Therefore, IL-5 and GM-CSF may be of crucial relevance in selective modulation of motility and chemotaxis of human EO.

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EOSINOPHIL MEDIATED VASCULITIS IN CONNECTIVE TISSUE DISEASE.

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Neutrophilic and lymphocytic vascular infiltration is commonly associated with vasculitis. We have identified 8 patients with *eosinophilic* necrotizing vasculitis in association with connective tissue disease (CTD), including Felty's syndrome (2), rheumatoid arthritis (2), systemic lupus erythematosus (3), and Sjogren's syndrome (1). Laboratory abnormalities included high titer rheumatoid factor, antinuclear antibody, hypocomplementemia and eosinophilia. Immunofluorescent localization of eosinophil granule major basic protein (MBP) and neutrophil elastase (NE) in lesional tissue showed marked MBP deposition in affected vessel walls with striking NE deposition surrounding the vessels peripheral to the MBP staining. In contrast, a control group of 9 patients with CTD-associated vasculitis without eosinophilia, showed marked NE deposition in involved vessels with scattered MBP staining. To analyze eosinophil-endothelial interactions, human pulmonary arterial endothelial cells (EC), either resting or stimulated with interleukin (IL)-1, were cultured overnight with eosinophils, in the presence of eosinophil activators including C5a, tumor necrosis alpha (TNF-α), platelet activating factor (PAF) and IL-5. Eosinophils activated with C5a (100 nM) adhered to IL-1 stimulated EC and EC viability was decreased; eosinophil activation by PAF (100 nM), TNF-α (100 ng/ml) and IL-5 (10 ng/ml) produced less EC destruction. Without IL-1 EC stimulation, no decreased EC viability and less eosinophil adherence were found. Ultrastructural examination showed eosinophil adherence to stimulated EC, phagocytosis of eosinophils by stimulated EC, rupture of EC membranes with lysis of organelles, and degenerated eosinophils in the cytoplasm of EC. The potent activity of C5a on eosinophils inducing chemotaxis, adherence to activated EC and degranulation with deposition of toxic granule proteins suggests a mechanism for vessel damage in CTD with eosinophilic vasculitis.

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C3A -BUT NOT C3A-DESARG- IS A POTENT ACTIVATOR OF THE RESPIRATORY BURST IN HUMAN EOSINOPHILS

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Complement activation leading to the formation of the anaphylatoxins C3a and C5a is suggested to play a major role in the propagation of various inflammatory skin diseases, particularly in bullous skin disorders. Whereas C5a is a potent activator of the respiratory burst in human eosinophils, the role of the anaphylatoxin C3a is poorly understood. The aim of this study was to investigate the potential role of human C3a -which was purified from zymosan-activated human serum by column chromatography- in the activation of the respiratory burst in eosinophils. The release of superoxide anion production of highly purified eosinophils from normal non-atopic blood donors was measured by lucigenin-dependent chemiluminescence (CL) using a 96-well chamber photon-imaging system. C3a induced a CL response in human eosinophils in a concentration-dependent manner, whereas C3a-desArg was inactive. However, the amount of superoxide anion production induced by C3a (13 fold at 500 ng/ml) was less than C5a (22 fold 100 nM).. The specificity of the response was confirmed by homologous desensitization after restimulation with C3a. In contrast, no cross-desensitization was observed upon stimulation with C5a. In addition, the effect of C3a was completely preserved in the presence of a monoclonal antibody against C3a. Blockade of the C5a receptor by the monoclonal antibody S5/1 totally inhibited the C5a-evoked CL-response, whereas the C3a response in the presence of S5/1 was unaffected. In summary, these results demonstrate for the first time that C3a, but not C3a-desArg, is a potent activator of the respiratory burst in human eosinophils. Therefore, the knowlegde about C3a and its controversial biological effect on human eosinophils in inflammatory skin diseases has to be reconsidered.

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THE T-CELL CHEMOTAXIN RANTES IS MORE THAN A CHEMOKINE - CHARACTERIZATION OF ITS EFFECT ON HUMAN EOSINOPHIL OXIDATIVE METABOLISM.

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Eosinophils were shown to play a major role in the allergic inflammatory process leading to the clinical symptoms of atopic dermatitis (AD). Only selected cytokines are capable of inducing a chemotactic response in eosinophils (EOs). In particular, the chemokine RANTES was recently shown to be a potent eosinophil chemotaxin. To get more insight into the role of RANTES in EO activation, the effect of RANTES and other chemokines on the oxidative metabolism of highly purified EO of normal non-atopic blood donors was investigated by assessment of functional as well as morphological criteria. RANTES, and, to a lesser extent, MIP-1α significantly induced the production of reactive oxygen species (ROS) by human EOs, whereas MCP-1, MIP-1β, and IL-8/NAP-1 had no significant effects. Besides of IL-8, none of the cytokines tested had any effect on PMN. Using scanning electron microscopy RANTES induced typical morphological changes, which were completely abrogated in the presence of cytochalasin B. However, RANTES stimulated only a part of the normal EOs. Based on functional and ultrastructural assay systems significant extracellular, but no intracellular H_2O_2 production was detected, which was completely inhibited by cytochalasin B. Separation of EOs by discontinous density gradients revealed the existence of two hypodense EO populations, one of them showing significantly reduced responses upon stimulation with RANTES. RANTES-induced production of ROS was almost completely inhibited by staurosporine, wortmannin and pertussis toxin. Based on these data it is evident that RANTES represents a potent EO-specific activator of human eosinophil oxidative metabolism. Besides of its chemotactic activity on T-cells and eosinophils, therefore, RANTES may be involved in the functional activation of eosinophils in the skin of AD patients, triggering their toxic capacities.

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METABOLISM AND URINARY EXCRETION OF ELAFIN, A SPECIFIC INHIBITOR OF HUMAN LEUKOCYTE ELASTASE. Volker Streit, Oliver Wiedow, Joachim Bartels, and Enno Christophers, Department of Dermatology, Christian-Albrechts-University, Kiel, Germany

Human leukocyte elastase (HLE) is a major proteolytic constituent of the azurophilic granules of polymorphonuclear leukocytes. It has been suspected that HLE contributes to the pathogenesis of diseases such as psoriasis, rheumatoid arthritis, ARDS and lung emphysema. Among the substrates of HLE are elastin, keratin, collagen type III and IV. Acid stable inhibitors of low molecular weight are able to interfere with the destructive protease activity at the tissue level in inflammatory conditions. Recently the two serine protease inhibitors elafin and antileukoprotease were found in human skin. For a better understanding of low molecular weight inhibitor metabolism we screened the urine of dermatological patients for protease inhibitors. The pooled urine of 5 patients with psoriasis (3) or erysipelas (2) was concentrated, acidified and subjected to cation exchange HPLC. Fractions with antiprotease activity were further separated via reversed phase C_8-HPLC, Poly LC-HPLC, and analytical reversed phase C_{18} and C_4-HPLC. With regard to retention times on HPLC and inhibition profile the purified inhibitor showed similiarity with elafin, an elastase specific inhibitor originally isolated from psoriatic scales. N-terminal sequence analysis of the first 28 residues revealed identity to elafin. While preproelafin deduced from DNA sequencing contains 117 residues, the elafin from psoriatic scales consists of 57 residues. The amino acid sequence of urinary elafin suggests that it has been cleaved at the lysine-glycine position beginning with the 7th of the known 57 elafin residues. Therefore it is one amino acid position (lysine) shorter than the smallest scale-derived elafin metabolite which starts at the 6th amino acid position of the original elafin. Further investigations are necessary to prove whether urinary elafin might be a useful marker in monitoring disease activity in various inflammatory conditions.

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HUMAN LEUCOCYTE ELASTASE CAN IMPAIR EPIDERMAL INTERCELLULAR COHESION. D.Ludolph-Hauser, O. Wiedow and E. Christophers, Department of Dermatology, University of Kiel, Faculty of Medicine, Kiel, Germany

Human Leucocyte Elastase (HLE) causes tissue destruction in various diseases. In psoriasis, contact dermatitis and atopic eczema where it is present in high concentrations on lesional skin however, its role is yet unclear. We now investigated the effect of HLE on intercellular cohesion in human epidermis. Freshly excised pieces of skin (25mm^2) were incubated in S-MEM-1099 medium with the dermal side after removal of the horny layer by stripping. HLE (3pmol-0.3nmol in 10µl 0.1M Sodium-acetate) was placed onto the epidermis from 15min to 4h. Experiments were performed at 37°C in an atmosphere of humid air containing 5% CO_2. Afterwards, specimens were fixed in formalin or glutäraldehyde and processed for lightmicroscopy or transmission electronmicroscopy. In contrast to previous authors who reported the effect of HLE being restricted to the dermo-epidermal junction, we found time- and concentration-dependent spongiosis followed by akantholysis in the malphigian layer. Cytoplasmic protrusions were noticed on each side of the desmosome-tonofilament-complexes which later lost contact with one or both of their neighbouring cells. There were no intradesmosomal splits. Interestingly, basal cells remained attached to the basal membrane without structural changes of hemidesmosomes. The distribution of spongiosis suggests a reaction with a protease-sensitive substrate being expressed in early stages of keratinocyte differentiation. Our results demonstrate that HLE alone can impair epidermal intercellular cohesion in vitro, resembling in vivo findings in eczema.

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THE T-CELL RECEPTOR REPERTOIRE OF LYMPHOCYTES INFILTRATING CUTANEOUS MELANOMA IS PREDOMINATED BY Vα SPECIFICITES PRESENT IN T-CELLS OF NORMAL SKIN. R Strohal[a], L Paucz[a], J Friedl[a], H Pehamberger[a] and G Stingl[a], DIAID[o], DGD[n], Dept. of Dermatology, Univ. of Vienna Medical School, Vienna, Austria

Lymphocytes infiltrating solid tumors (= tumor infiltrating lymphocytes = TIL) can be propagated with IL-2 and are then capable of lysing autologous tumor targets in a class I-restricted manner. As the specificity of these $CD8^+$ T-cells is determined by their T-cell receptor (TCR) configuration, we decided to define the Vα TCR repertoire within 5 naevocellular nevi and 24 melanoma specimens using the reverse polymerase chain reaction and to compare these data with the TCR expression pattern of unaffected peritumoral (n=2) and normal human skin (n=5) and one unaffected axillary lymph node. As compared to lymph node T-cells, which gave positive results for 15 of the 18 Vα families, normal human skin showed a substantial, albeit limited heterogeneity of Vα specificities with an average number of 9.71 different Vα gene segments expressed. Moreover, by analysing the individual quantitative level of expression of each family, we found a very homogeneous Vα pattern as 9 Vα families were amplified in 100% of the skin specimens tested (Vα1, Vα5, Vα7, Vα11, Vα12, Vα13, Vα14, Vα15, Vα16), 3 Vα gene segments were irregularly expressed and all other Vα genes were missing. In contrast, the Vα repertoire within cutaneous melanoma lesions was significantly more restricted (mean of Vα expression: 3.86; $p < 0.001$) with a predominance of only 3 Vα families (Vα13/83.6%, Vα15/75%, Vα16/75%), which were recruited from the skin-associated TCR Vα repertoire. No evident association was found between the usage of Vα regions and the site of excision, the histopathologic diagnosis, the prognostic parameters and the progression of the tumors. These data (1) demonstrate a restricted usage of TCR genes in lymphocytes infiltrating melanoma lesions, and (2) indicate that TIL target only a limited number of melanoma-associated antigens.

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DIFFERENT MIGRATION PATTERNS OF LANGERHANS CELLS IN SKIN LYMPH DERIVED FROM IRRITANT AND ALLERGIC CONTACT DERMATITIS. Christoph U. Brand, Thomas Hunziker, Alain Limat, Lasse R. Braathen, Dermatological Clinic, University of Berne, Switzerland

To monitor the kinetics of Langerhans cells (Lc) during contact dermatitis a peripheral lymph vessel draining a defined skin area on the foot was cannulated in four healthy human volunteers. In two of them an irritant contact dermatitis was induced in the drained skin area by application of 10 % sodium lauryl sulphate, in two others, one of them previously sensitized, the contact allergen diphenylcyclopropenone (DPCP) was applied. In the irritant contact dermatitis a large increase of Lc was demonstrated in the lymph in the late phase and even after resolution of the skin reaction. In the induction phase of the DPCP allergic contact dermatitis, however, an impressive output of Lc became evident during the first 24 hours after application of DPCP, whereas in the elicitation reaction the main Lc output occurred in the intermediate and late phase of the skin reaction. Ultrastructural analyses of Lc in lymph from the late phase of an elicitation reaction revealed only few Birbeck granules, and immunohistochemically these cells were $CD1a^+$,$HLA\text{-}DR^+$, $ICAM\text{-}1^+$ and in part also $LFA\text{-}3^+$. These results provide direct evidence that the migration of Langerhans cells is a major feature of irritant and allergic contact dermatitis in humans, and that the time course patterns of the migration are different in the various reactions.

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CHARACTERIZATION OF THE HUMAN KU AUTOANTIGEN EXPRESSED BY BACULOVIRUSES. M.ONO, R.D.Sontheimer, P.W.Tucker, J.Donald Capra, Depts. of Microbiology and Dermatology, U.T.Southwestern Med.Center, Dallas, TX,

Autoantibodies to Ku are produced by patients with rheumatic diseases. Ku is a nuclear autoantigen heterodimer (HD) consisting of two subunits (p70 and p86). This polypeptide complex has been shown to bind to DNA in vivo. While there have been many attempts to analyze the interaction of Ku with DNA, the nature of this interaction is still unclear. To better understand the molecular basis of Ku, we expressed the p70 and p86 subunits separately and assembled them into HDs using a Baculovirus system. These HDs were then used to further analyze the DNA binding property of Ku. Two recombinant Baculoviruses (AcNPV2.1, VBB2-86Ku) were developed. Sf9 cells were infected with each or both of these constructs. The recombinant Ku subunits were harvested at 72h post-infection and prepared for assay. HDization and DNA binding of the Ku subunits were analyzed with co-immunoprecipitation and an electrophoretic mobility shift assays. HDized Ku was expressed in cells co-infected with both AcNPV2.1 and VBB2-86Ku, however, separately expressed subunits did not assemble into HDs in vitro. The HDized Ku bound linear double-stranded DNA (dsDNA) efficiently without sequence specificity. Neither subunit alone could bind DNA. Furthermore, Ku HDs could not bind supercoiled DNA, nicked circular DNA, nor linear single-strand DNA efficiently in vitro. In addition, anti-Ku autoantibodies blocked the binding of Ku to DNA. These results suggest that a) HD formation is required for Ku to bind DNA, b) Ku binds preferentially to linear dsDNA under physiological conditions, and c) HDization of Ku may require co-translation of the individual subunits or an intracellular environment. This expression system should facilitate the delineation of the normal cellular function of Ku as well as autoimmune response to Ku.

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WILD TYPE P53 AND METALLOTHIONEIN ARE EXPRESSED SIMULTANEOUSLY IN UV IRRADIATED SKIN: A POSSIBLE LINK TO PHOTOCARCINOGENESIS. B. Jasani*, A. Anstey, R. Marks, C.C. Long and A.D. Pearse, Departments of Dermatology and *Pathology, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, U.K.

There has been considerable speculation over the roles of mutant P53 gene product (P53GP) and metallothionein (MT) in tumorigenesis although little is known of their normal expression and physiological roles. In the present study we have used immunocytochemical methods to characterize the expression of MT and P53GP in (a) non-irradiated normal buttock and axillary skin and (b) after 3 MED UV irradiation at 300 nm and (c) after 15 MED irradiation but protection with a SPF15 sunscreen. A standard two stage indirect immunoperoxidase method was used with the 18DI antibody for wild type P53 and the E-9 antibody directed at a conserved epitope of MT-1 and MT-2 isoforms. In all 6 paired samples from buttocks there was concurrent expression of P53GP and MT in irradiated non-UV-protected skin compared to non-irradiated skin. In UV-protected but irradiated skin MT expression was negative but P53 expression was either negative or weakly and patchily positive. MT was expressed in basal keratinocytes in irradiated UV-protected and control skin but appeared more strongly expressed in irradiated unprotected skin. MT is thought to protect against UVR by scavenging reactive oxygen species which cause the initial cell damage. Wild type P53 may also protect against UVR induced DNA damage. We have demonstrated simultaneous expression of both proteins after UVR irradiation and speculate that their induction is a protective mechanism preventing DNA damage. Occurrence of the P53 suppressor gene mutation occurring simultaneously with induction of metallothionein may assist survival of damaged cells with subsequent clonal expansion of abnormal cell types.

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CHEMOTACTIC MIGRATION OF DENDRITIC EPIDERMAL T CELLS TOWARD PAM 212 KERATINOCYTE SUPERNATANTS. BS Chung, PR Bergstresser, A Takashima. UT Southwestern, Dallas, TX, USA and Chosun Univ., Kwangju, Korea

Mouse epidermis contains a population of γδ T cells, termed dendritic epidermal T cells (DETC). We observed recently that keratinocytes (KC) provide an adhesion substrate for DETC and that they support their survival and growth by secreting IL-7. The present study was conducted to address the mechanisms by which DETC migrate into the epidermis, reasoning that KC may be a source of chemotactic activity. Using a modified Boyden chamber method, short-term DETC lines were tested for their migratory capacities toward Pam 212 KC culture supernatants; migration of ^{35}S-labeled DETC from upper chambers toward test samples in lower chambers was assessed by counting radioactivity. All DETC lines tested displayed rapid (within 60 min) and marked (>50%) migration toward Pam 212 KC supernatants, but not toward 3T3 fibroblast supernatants. Addition of Pam 212 supernatants to upper chambers completely blocked migration, suggesting its chemotactic nature. The majority of cells that had migrated into Pam 212 supernatants expressed the Vγ3 T cell receptor, thus verifying their identity as DETC. The chemotactic activity was: 1) produced by Pam 212 KC even in the absence of serum; 2) greater than 12 kD in size; 3) temperature- and pH-labile; 4) trypsin-sensitive; and 5) precipitated by 60-100% ammonium sulfate. Cytokines known to be produced by KC (e.g., IL-1α, IL-8, GM-CSF and TNFα) failed to mediate DETC migration, when added to the lower chambers, or to inhibit migration toward KC supernatants, when added to upper chambers. These results support our hypotheses that KC facilitate the residence of DETC in epidermis by secreting unique chemotactic factors, by providing adhesion substrates, and by elaborating specific growth factors.

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IN VITRO HAPTEN TREATMENT STIMULATES THE MIGRATION OF HUMAN EPIDERMAL LANGERHANS CELLS. Yasunobu Kobayashi, Marie-Jeanne Staquet, Colette Dezutter-Dambuyant and Daniel Schmitt, INSERM U.346, Hôpital Ed.-Herriot, Lyon, France.

Previous studies have shown that epicutaneous antigen-application induces a significant accumulation of antigen-bearing dendritic cells in draining lymph nodes, suggesting that epidermal Langerhans cells (LC) are capable of migrating and carrying foreign antigens from epidermis to lymph nodes. The aim of the present study was to investigate if *in vitro* hapten application modifies the motility of LC. In order to study whether haptens could induce this migration, especially if hapten application triggers LC to leave the epidermis, an *in vitro* migration assay was performed to assess the invasive capacity of hapten-modified LC through a reconstituted basement membrane (Matrigel). Freshly isolated human LC-enriched suspensions (8-15%) were treated with Picrylsulfonic acid (TNBS) for 10 min at 37°C, washed and plated onto Matrigel-coated polycarbonate filters (Nuclepore, 8μ pore size) placed in modified Boyden-chambers. The lower compartment of Boyden-chambers was filled with human dermal fibroblast-conditioned medium. After incubation (20hr, 37°C), the filters were fixed and stained with anti-CD1a and -HLA-DR monoclonal antibody, and the number of invading cells present on the lower filter surface was counted.

Little invasion was observed by non-treated LC. By contrast, TNBS-modified LC showed a dose-dependent increased invasion. The number of invading cells decreased in the absence of fibroblast-conditioned medium, suggesting that some fibroblast-derived chemoattractants stimulate the directional migration of LC. Invading LC strongly expressed HLA-DR, although they showed a decreased CD1a expression. Our present results show that the hapten-LC contact acts as a trigger to initiate the motility of LC; this may in turn enable LC to leave epidermis for dermis and migrate to lymph nodes.

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CHEMOTACTIC FACTOR(S) OF LANGERHANS CELLS IN CONTACT SENSITIVITY. Sayuri Yamazaki, Ichiro Katayama, Hiroo Yokozeki, Takahiro Satoh, Kiyoshi Nishioka, Department of Dermatology, Tokyo Medical and Dental University, School of Medicine, Tokyo, Japan.

Langerhans cells (LCs) migrate to the regional lymph node(LN) after hapten application and function as antigen presenting cells. Regulatory molecules of LC migration to the regional LN is still obscure. We focused to obtain lymph node cell production of such a chemotactic factor(s). Culture supernatant of LN cells obtained from TNCB painted mice (TNP-sup) was examined for its chemotactic activity for LCs, using a modified Boyden chamber method. The TNP-sup enhanced LC migration in a chemotactic manner. About 90 % of migrated epidermal cells were LCs. LC migration was partially inhibited by anti-ICAM-1 and anti-LFA-1 antibodies, but not by anti-TNF-α and anti-GM-CSF antibodies. HPLC analysis of the TNP-sup showed that the chemotactic activity was eluted as molecule(s) between 45 and 68 kD. These data indicate that LN cells produce a chemotactic molecule(s) for LCs which is different from TNF-α and GM-CSF in contact sensitivity.

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MIGRATION OF DENDRITIC EPIDERMAL T CELLS (DETC) ; LY48 AND TNF-α ARE RESPONSIBLE FOR THE MIGRATION OF DETC TO THE EPIDERMIS IN AN IN VITRO ORGAN CULTURE MODEL SYSTEM. [1]Atsushi Saitoh, [1]Nami Yasaka, [2]Anthony A. Gaspri, [1]Masutaka Furue and [1]Kunihiko Tamaki, [1]Department of Dermatology, Yamanashi Medical University, Yamanashi, Japan, [2]Division of Dermatology, Unversity of Rochester, New York, USA.

DETC are originally thought to be derived from bone marrow. Recent evidence suggests, however, $\gamma_s\delta_1$ TCR bearing DETC originate from day 16 fetal thymic cells. The behavior of these cells are still obscure. To understand the migratory capability of DETC, we developed an in vitro skin organ culture model. First, emigration of DETC from the epidermis was examined. Ear skin from C3H/He mice was separated into two parts and incubated for 3days with dermal side down. It was found that DETC emigrate from the epidermis into the dermis and then migrated out of the skin into the culture medium. Next, immigration of DETC into the epidermis was examined. For this, DETC were depleted by daily application of clobetazole propionate solution (CP) topically onto the ears of C3H/He mice. Seven days later, ear skin was separated and cultured at dermal side up with syngeneic epidermal cell (EC) suspension with special device for 3days. It was found that 1) DETC immigrated into the DETC depleted epidermis remarkably compared to normal epidermis, 2) the migratory capability of DETC was directly proven by the biolabelling technique with PKH-26. Moreover, when various antibodies (Ab) were added in these culture, it was found that monoclonal Ab (MoAb) against S11, mouse leukosialin Ly48 and TNF-α, inhibited the immigration significantly, but not completely. The other Ly48 MoAb (S15, S7, 3E8), however, failed to inhibit the immigration of DETC into the epidermis. MoAb against CD44, LFA-1, IL-6, IL-2, fibronectin and laminin did not affect their immigration, either. These results suggest that DETC retain the potential of migration and that S11, leukosialin expressed on DETC, and keratinocyte derived cytokine TNF-α are partially responsible for the immigation of DETC into the epidermis. Thus, this in vitro model could be used to further analyze the migration of DETC in vitro.

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DISTINCT SIGNALING PATHWAYS ARE ACTIVATED BY IL-2 AND BY IL-7 IN DENDRITIC EPIDERMAL T CELLS. K Ariizumi K, PR Bergstresser, A Takashima. Dept. of Dermatology, UT Southwestern Medical Center, Dallas, TX, USA.

Mouse epidermis contains a population of γδ T cells, termed dendritic epidermal T cells (DETC). We observed recently that the growth of DETC is supported by DETC-derived IL-2 and by keratinocyte (KC)-derived IL-7. To study the mechanisms by which DETC respond to these cytokines, we analyzed IL-2- and IL-7-dependent signaling pathways in the 7-17 DETC line. Binding assays using biotinylated rIL-2 and rIL-7 indicated that IL-2 and IL-7 bind independently to DETC. Proliferative responses to IL-2 and to IL-7 were blocked equally by methyl 2,5-dihydroxycinnamate and by Genistein, suggesting that tyrosine kinase cascades are involved in both pathways. Neither IL-2 nor IL-7 induced detectable Ca^{2+} influx, as measured with Indol-AM. We then analyzed mRNA expression of early immediate genes, whose products are known to serve as nuclear mediators by binding to promoter regions of functional genes. Northern blot analyses demonstrated that c-myc mRNA was upregulated (5 to 10-fold in 30 min) after stimulation with IL-2 (10 ng/ml) or with IL-7 (10 ng/ml). Significant upregulation of c-jun, jun-B, jun-D and c-fos was also induced by IL-2 and by IL-7. In contrast, Egr-1 mRNA was greatly upregulated (>50-fold) by IL-2, but not by IL-7. Kinetic experiments demonstrated that these responses occurred rapidly, with significant upregulation detectable within 10 min, and peaking at 30-60 min. These results indicate that the jun/fos and the c-myc pathways are activated by either IL-2 or IL-7, whereas the Egr-1 pathway is activated only by IL-2. We propose that IL-2 and IL-7 utilize different signaling pathways in the activation of DETC.

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REGULATION OF LANGERHANS CELL (LC) ANTIGEN PRESENTATION BY CALCITONIN GENE-RELATED PEPTIDE (CGRP) AND GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (GM-CSF). R.D. Granstein, J. Hosoi, S. Bruyers, G.F. Murphy* and A. Asahina. MGH/Harvard Cutaneous Biology Research Center, Massachusetts General Hospital, Boston, MA, USA and *Dept. of Dermatology, Univ. of Pennsylvania, Philadelphia, PA, USA

CGRP inhibits LC antigen-presenting function and CGRP-containing nerves are anatomically associated with LC. GM-CSF augments LC antigen-presenting function in a number of assays. To further define the activity of CGRP on LC, its ability to modulate LC presentation of chick ovalbumen (cOVA) to the T-T hybridoma DO11.10 was studied with and without simultaneous exposure to GM-CSF. Epidermal cells (EC) were prepared from CAF_1 mice and pulsed with cOVA or an immunogenic fragment of cOVA recognized by DO11.10. EC were exposed to CGRP (100 nM), GM-CSF (50 U/ml), both or neither for 2 hr before antigen pulsing and during the period of antigen pulsing. Cells were then washed and 10^5 cells cultured with 1 x 10^5 DO 11.10 cells in 200 μl of complete medium. After 24 hr, supernatants were harvested and assayed for IL-2 content by stimulation of the IL-2-dependent line CTLL. CGRP inhibited presentation of cOVA and the immunogenic fragment, both with and without the simultaneous presence of GM-CSF [For cOVA: EC-8720±1130(SEM) CPM, EC (CGRP)-4690±800 (p=0.03); EC (GM-CSF)-20310±1530, EC (GM-CSF+CGRP)-9710±1270 (p=0.001). For the fragment: EC-25437±2122, EC (CGRP)-11503±594 (p<0.001); EC (GM-CSF)-38533±1723, EC (GM-CSF+CGRP)-22237±1976 (p<0.001)]. A CGRP antagonist ($CGRP_{8-37}$) was employed as a control and failed to inhibit presentation in this assay. In other experiments, EC were cultured for 48 hr with CGRP, GM-CSF, both or neither, followed by harvesting of non-adherent cells, enrichment for LC by density centrifugation and presentation of the immunogenic fragment of cOVA to DO 11.10 cells. CGRP also inhibited antigen presentation utilizing this protocol. These findings show that CGRP can inhibit antigen presentation by LC even in the presence of a concentration of GM-CSF that augments antigen presentation in some systems. These data suggest that epidermal nerves may regulate LC function through release of CGRP.

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ORIGIN, BINDING CHARACTERISTICS AND GLYCOFORM-SPECIFIC IGE-BINDING OF THE HUMAN IGE-BINDING PROTEIN IN LANGERHANS CELLS. A. Wollenberg[1], E Sander[1], FT Liu[2], and T Bieber[1], [1] Dept. of Dermatology, University of Munich, Germany, [2] Scripps Research Institute, La Jolla, CA, USA

Recently, the so-called IgE-binding protein (εBP), a monovalent ß-galactoside binding lectin, has been reported in normal human skin and especially on the surface of Langerhans cells (LC); in the present work we investigated the origin of εBP found on LC and its binding characteristics to LC and different IgE-glycoforms. First, εBP expression on LC varied considerably interindividually, as determined by flow cytometric analysis, suggesting an εBP-expression on LC dependent on individual environmental cell conditions. Immunoblot analysis of human normal keratinocytes (HNK) and purified LC disclosed an identical 33 kDa protein. Detection of εBP mRNA by Northern blot remained negative for LC but was positive for HNK leading to the hypothesis that the latters may represent a source of εBP for LC. LC incubated in conditioned medium from HNK or HaCaT cells showed an increased εBP-reactivity of LC in flow cytometry, implicating an active εBP-release by HNK followed by binding to LC-surfaces. Furthermore, a dose dependent increase of the εBP-binding to LC was observed by using recombinant human εBP, thus revealing numerous additional potential εBP-binding sites. Lectin type binding was confirmed by removal of εBP from the LC-surface with lactose. Short time culture increased the εBP-expression on LC while their potential binding sites were reduced. Incubation of LC with IgE in the presence of εBP increased the binding of low sialylated IgE, whereas IgE-binding of highly sialylated IgE was reduced. IgE-binding via εBP-dimers might explain the first effect, the second one might be due to a direct εBP-FcεRI-interaction. We conclude that (i) KC actively release εBP; (ii) εBP binds via its lectin property on the surface of LC glycoproteins; (iii) εBP-binding site(s) on LC progressively disappear(s) in vitro and (iiii) εBP modulates the IgE-binding capacity of LC in vitro towards low sialylated IgE-species.

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IN HUMAN LANGERHANS CELLS THE "CONTINUOUS ENDOSOMAL RETICULUM" COMPRISES BOTH BIRBECK "GRANULES" AND "CORED TUBULES" AND APPARENTLY PARTICIPATES IN A RECYCLING PATHWAY. D. Hanau[(1)], H. de la Salle[(1)], T. Bieber[(1)], M.-E. Esposito-Farese[(1)], C. Gachet[(2)], M. Fabre[(3)] and J.-P. Cazenave[(2)], [(1)]Laboratoire d'Histocompatibilité and [(2)]INSERM U.311, Centre de Transfusion Sanguine, Strasbourg; and [(3)]Service Central de Microscopie Electronique, Université Louis Pasteur, Strasbourg, France.

In 1990, Hopkins *et al.* (Nature, 346 : 335-339) reported that the early part of the endocytic pathway is composed of a branching reticulum along which endosomes move, as do ligand-receptor complexes. This "continuous endosomal reticulum" appears to be labile, sensitive in particular to the chemical fixatives used to prepare samples for electron microscopy, which cause it to break up into short tubulo-vesicular elements. In the present electron microscopic study we followed, in untreated and monensine-treated (20 mM) Langerhans cells (LC), the internalization at 37°C over 25 min of gold-labeled anti-CD1a monoclonal antibody. On this occasion we had the opportunity to observe, near the cell surface, images of continuity between gold-labeled coated vesicles and a tubular reticulum and between gold-labeled reticulum and endosomes. Moreover, in the cell center near the centriole, we observed the presence of gold-labeled tubules often branched to form irregular interconnected networks. These tubules were continuous with gold-labeled endosomes. They often appeared, on longitudinal sections, to present an inner central thin line with no periodicity and, on cross sectional profiles, to be circular with a central dot. They thus presented the ultrastructural characteristics described in mice for "cored tubules" (J. Electron Microsc, 32 : 197-206, 1983). However, in some zones, the normally irregular contours of the tubular elements of the reticulum became suddenly straight with appearance of a central striated zone. The tubules then present the pentalaminar structure characteristic of Birbeck granules. Moreover, in places gold-labeled coated pits appeared to bud from tubular elements of the reticulum. Thus, our observations (i) described for the first time the presence of "cored tubules" in epidermal human LC, (ii) demonstrated that at least part of the cytoplasmic Birbeck "granules" are part of the "continuous endosomal reticulum" and (iii) suggest that the latter may be involved in the recycling pathway of CD1a antigens.

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FUNCTIONAL HUMAN EPIDERMAL LANGERHANS CELLS THAT LACK BIRBECK GRANULES AM Mommaas, AA Mulder, F Koning and BJ Vermeer, Depts of Dermatology and Immunohaematology and Bloodbank, University Hospital Leiden, Leiden, The Netherlands

Since the first description of the Birbeck granule (BG) as a cytoplasmic organelle, specific for epidermal Langerhans cells (LC), many papers have appeared in the literature addressing the origin and function of this organelle. Some propose that BG arise in the Golgi area, migrate towards the cell periphery and release their content in the extracellular space. A majority, however, supports the theory that BG arise from the cell membrane as part of the receptor-mediated endocytosis pathway. It has been claimed that BG are actively involved in the intracellular traffic of CD1a antigens or that they participate in the antigen-processing/presenting functions of the LC. During a study in which the role of UVB on the human epidermal LC function and morphology was determined, we identified a healthy caucasian 29-year old male, whose LC completely lacked the presence of BG. This was repeatedly observed using skin biopsies taken from several places of the body during a period of 2 years. Despite the complete lack of BG, the LC displayed all the usual morphological characteristics, such as a lobulated nucleus and clear cytoplasm, devoid of tonofilaments, and were HLA class II and CD1a positive. His epidermal cells showed normal responses in the mixed-epidermal-cell-lymphocyte-reaction, which after 4 weeks of UVB therapy were strongly reduced, similar as was found for the other participants of the experiments.

Our findings suggest that in normal human epidermis, BG represent a rudimentary-like organelle, and they are not a prerequisite for receptor-mediated endocytosis or antigen processing and presentation.

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FUNCTIONAL EXPRESSION OF THE COSTIMULATORY MOLECULE B7 ON ACCESSORY CELLS FOR MHC CLASS I-RESTRICTED IMMUNE RESPONSES. Adelheid Elbe, *Hans Reiser, Dirk Strunk, Susanne Schreiber and Georg Stingl, DIAID, Dept. of Dermatol., VIRCC, Univ. of Vienna Med. School, Vienna, Austria and *Div. of Lymph. Biology, Dana-Farber Cancer Inst., Boston, MA

We have generated a dendritic cell line from murine fetal skin displaying the $CD45^+$/MHC class I^+/MHC class II^-/$Fc\gamma RII^+$/$CD11b^+$/$F4/80^+$/TCR^- phenotype. We have previously shown that this cell line is capable of activating naive, allogeneic $CD8^+$ T cells in a MHC class I-restricted fashion. This finding was surprising in that other MHC class I^+/II^- cell types (P388D1, L929, PAM 212, freshly prepared keratinocytes, peritoneal macrophages) were not able to do so. Antigen-specific T cell stimulation requires the delivery of both the antigenic and a costimulatory signal such as the B7/BB1 antigens which are strongly expressed on cells of the Langerhans cell/dendritic cell family. Using a recently produced murine anti-B7 mAb, we found that 80/1 cells displayed anti-B7-reactivity similar in magnitude to that seen with cultured Langerhans cells. In contrast, P388D1, L929 and PAM 212 cells were negative. More importantly, continuous presence of anti-B7 mAb in the cocultures resulted in a 99% inhibition of MLR. Whereas pretreatment of 80/1 cells with the anti-B7 mAb led to an 82% inhibition of the allogeneic MLR, pretreatment of responder $CD8^+$ lymph node T cells with anti-B7 mAb had no effect.

In summary we have shown that the additional presence of a costimulatory molecule makes non-immunogenic, MHC class I-bearing cells capable of eliciting a primary productive immune response. These findings have important clinical implications concerning the development of vaccination strategies against endogeneous pathogens.

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DIFFERENT E-CADHERIN EXPRESSION BY $V\gamma5$ TCR^+ AND $V\gamma5$ TCR^- γ/δ TCR^+ T CELLS IN THE MURINE SKIN. H Ozawa, S Aiba, S Nakagawa and H Tagami. Department of Dermatology, Tohoku University, Sendai, Japan

Recently we have reported the presence of two different populations of γ/δ TcR^+ T cells in the murine skin. One is $V\gamma5$ TcR^+ T cells which have been well known as $Thy\text{-}1^+$ dendritic epidermal T cells. The other is $V\gamma5$ TcR^- T cells, which migrate with Langerhans cells from the organ cultured of murine skin. Analysis of TcR of the latter by PCR demonstrated that Vγ1, Vγ2, and Vγ4 were used. The purpose of this study was to examine the expression of adhesion molecules by these two different populations of cutaneous γ/δ TcR^+ T cells. We enriched $V\gamma5$ TcR^+ T cells from murine epidermal cell suspensions by using anti-Ly5 antibody and anti-mouse Ig-coupled magnetic beads. $V\gamma5$ TcR^- T cells were obtained by collecting migrating cells from organ cultured skin. These two different γ/δ TcR^+ cells were cultured with murine fibroblast-like cell line, 12E2, which we have recently established, in the presence of IL-2. Two weeks later, we obtained enough numbers of γ/δ TcR^+ T cells to analyze the surface phenotype and their expression of adhesion molecules. Flow cytometry was performed using the antibodies to γ/δ TcR, $V\gamma5$ TcR, vitronectin receptor (α chain), VLA-4 (α chain), and E-cadherin. Both $V\gamma5$ TcR^+ and $V\gamma5$ TcR^- T cells expressed vitronectin receptor (α chain), but neither expressed VLA-4 (α chain). Interestingly, $V\gamma5$ TcR^+ T cells expressed E-cadherin, while $V\gamma5$ TcR^- T cells did not. The different expression of E-cadherin between two different populations of cutaneous γ/δ TcR^+ T cells may decide their localization in the skin.

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DISTRIBUTION AND HAIR CYCLE-DEPENDENT NUMERICAL CHANGES OF GAMMA-DELTA T CELLS IN MURINE SKIN. Udo Hofmann, Ralf Paus, Stefan Eichmüller and Beate M. Czarnetzki. Dpt. of Dermatology, University Hospital R. Virchow, Freie Universität, D-1000 Berlin 65, Germany

Gamma-delta T cells (gdTC) are recognized as the predominant intraepidermal T cell population in murine skin, although their physiological functions are still unclear. Little is known on the exact distribution of gdTC in the other epithelial skin compartments of normal mice. Using the C 57 BL-6 mouse model for hair research and selective gdTC-receptor antibodies in immunohistology (alkaline phosphatase technique), longitudinal cryostat sections of whole back skin in all the different stages of the depilation-induced hair cycle were analyzed morphometrically for the distribution and number of gdTC. We found that, during the entire hair cycle, V gamma 3 (Vg3)-immunoreactive gdTC are restricted to the epidermis and to the epithelial hair bulb in and distal to the bulge area. No gdTC were seen in the cycling, proximal portion of the hair bulb or in the sebaceous glands. Since there was no phenotypical difference of Vg3+ cells in the epidermis and in the hair follicle, Vg3+ dendritic epidermal T cells (DETC) and intrafollicular, Vg3+ cells may best be summarized under the term dendritic intraepithelial T cells (DIETC). During early anagen development, when intraepidermal keratinocyte proliferation after depilation is maximal, the number of pan-gdTC receptor-positive cells increased significantly ($p<0.005$) in the interfollicular epidermis and the suprainfundibular portion of the hair bulb, while it decreased in the bulge region ($p<0.005$). Since gdTC are thought to migrate into the skin only during embryogenesis and early postnatal life, this finding in adolescent mice suggests hair cycle-dependent, differential intraepithelial proliferation of gdTC in murine skin. We advocate to employ only skin of defined hair cycle stages in immunological studies on murine skin, and outline uses of this model for dissecting the still unclear functions of gdTC in skin and hair biology.

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ISOLATION AND CHARACTERIZATION OF A T CELL LINE FROM THE GUT OF A PATIENT WITH DERMATITIS HERPETIFORMIS (DH); EVIDENCE FOR RESTRICTED T CELL RECEPTOR (TCR) Vß EXPRESSION. ML Keogh, JAP Wilson, R Streilein, RP Hall, Dermatology and Gastroenterology, Duke Univ Med Center, Durham, NC, USA.

DH is associated with an asymptomatic, gluten sensitive enteropathy (GSE) characterized in part by a T cell infiltrate in the small bowel mucosa. The factors controlling this inflammatory response in the gut are not known. We have isolated T cell lines from the gut of 3 DH patients and in 1 patient expanded a T cell line. This expanded T cell line has been analyzed for T cell phenotype, antigen and mitogen responses, and for TCR Vß family utilization. T cells were derived from gut biopsies of patients with DH during gluten challenge. Two T cell lines were expanded with IL2 and PHA. FACS analysis revealed 97% CD3, 98% CD4, 1 - 3% CD8, 93- 97% CD45 RO, 39 - 45% Leu- 8 positive. T cell lines proliferated to PHA (SI = 121) and anti-CD3 (SI = 28) but not to gliadin or ß-lactoglobulin (SI < 1.0). TCR Vß utilization was determined using semiquantitative reverse transcription-polymerase chain reaction with primers specific for 20 different Vß families in 1 T cell line and in the peripheral blood lymphocytes (PBLs) from the patient before and during gluten challenge. No significant difference in Vß utilization in PBLs was noted, with individual TCR Vß family utilization ranging from 0 - 14%. In the T cells isolated from the gut a markedly increased usage of Vß4 (60%) and Vß11 (20%) was detected when compared to PBLs during gluten challenge (Vß4 = 10%, Vß11 = 1%), demonstrating a restricted expression of TCR in the gut T cell lines. PHA stimulation of PBLs from this patient did not result in a similarly restricted TCR Vß expression. These data demonstrate that T cells can be cultured from small bowel and suggest a restricted TCR Vß family utilization in the gut of patients with DH which may have important implications in understanding the pathogenesis of DH.

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ANDROGEN RECEPTORS IN PERIPHERAL MONONUCLEAR LEUCOCYTES ARE DECREASED IN MALE PATIENTS WITH LUPUS ERYTHEMATOSUS AND ARE SPECIFICALLY EXPRESSED IN CD4-POSITIVE T-LYMPHOCYTES. Michael Meurer*, Jörg C.Prinz*, Ursula Keller, Ursula Kuhnle, Department of Dermatology*; Pediatric Endocrinology, Children' Hospital,University of Munich 80337 Munich, Germany

Androgen receptors (AR) have recently been demonstrated in intact circulating peripheral mononuclear leucocytes (PML). In healthy adult men testosterone bound to a single class of receptors with an affinity of 1±0,5 nM and a mean capacity of 203 receptor sites/cell (range 120-270 sites/cell, n=20). The specifity data were similar to those reported for genital fibroblasts except for equal binding of testosterone and dihydrotestosterone (=100%), estradiol (=12%), progesterone (=6%). In PML from patients with lupus erythematosus (5 x SLE, 3 x SCLE, 4 x DLE) androgen receptors were significantly reduced ($p<0.005$): mean AR concentration per cell was 98 (SD 74.3, range 38-220 sites/cell, n=12). There was no correlation between AR concentrations in PML and type of lupus erythematosus or treatment, serum levels of testosterone or CD4/CD8 ratios.

In order to determine how AR are distributed in the T-cell subpopulations binding studies were performed after separation in CD4 and CD8 positive cells by the direct monoclonal antibody rosetting technique using ox erythrocytes coated with monoclonal CD4- or CD8-antibodies. Rosette forming cells were depleted from PML via Ficoll density centrifugation. AR could only be detected in the CD8- depleted subpopulation while the CD4-depleted cell fraction was consistently negative (3 healthy men, 2 male patients with SLE). These results are the first evidence that androgen receptors are specifically expressed in CD4-positive T-lymphocytes. Together with earlier reports that estrogen receptors are present only in CD8-positive cells, these results may have important implications for the cellular immune response in lupus erythematosus and its sex specific prevalence.

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PERIPHERAL BLOOD MONOCYTES EXPRESS THE HIGH-AFFINITY IgE RECEPTOR, FcεRI. Dieter Maurer, Edda Fiebiger, Oliver Kilgus, Bärbel Reininger, Kenichi Ochiai, Jean-Pierre Kinet and Georg Stingl. DIAID, Dept. of Dermatology, Univ. of Vienna Med. Sch., Vienna, Austria and Mol. Allergol. and Immunol. Sect., NIAID, NIH, Rockville, MD, USA

In allergic diseases, IgE-dependent activation of monocytes/macrophages (Mø/MP) appears to play an important role in the initiation of inflammatory reactions. With regard to the expression of IgE-binding structures on Mø/MP, numerous studies have suggested that the inducible form of the low-affinity IgE receptor, CD23, constitutes the unique acceptor site for IgE on these cells.

Based on our previous observation that cellular binding of monomeric IgE via the high-affinity IgE receptor FcεRI is not a unique property of basophils and mast cells, but also occurs with epidermal Langerhans cells, we have re-evaluated the putatively exclusive role of CD23 for IgE-binding to Mø/MP. Using two-color flow cytometry we made the observation that mAb 15-1, directed against the FcεRI α-chain, reacts with freshly isolated $CD14^+$ PB-MNC in a proportion of adult volunteers. The specificity of this reactivity was confirmed 1) by the reduction/abrogation of mAb 15-1 $F(ab')_2$ binding to Mø following preincubation of the cells with graded doses of monomeric, monoclonal human IgE but not of monomeric, monoclonal human IgG or of Fc fragments derived from human serum IgG and 2) by the demonstration that binding of monomeric IgE to PB-Mø was completely blocked by preincubation of the cells with $F(ab')_2$ fragments of the anti-FcεRIα mAb 15-1, but not with anti-CD23 (mAb MHM6) or binding/non-binding isotype-matched control mAbs. The detection of FcεRIα- and FcεRIγ mRNA in PB-Mø indicated that monocytes synthesize FcεRI. Finally, it apears that monocyte-derived FcεRIα- and FcεRIγ-chains have MW characteristics identical to those of basophils and of CHO cells transfected with FcεRIα/γ constructs. Taken together, these results demonstrate (i) that human PB-Mø express FcεRI moieties and (ii) that this receptor is the critical Mø binding structure for monomeric IgE. Our further observation that the expression of this receptor is greatly upregulated in atopic individuals suggests that FcεRI expression on Mø may be pathogenetically linked to the development/perpetuation of atopic disorders.

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IMMUNOLOGICAL CHARACTERISTICS OF ULTRAVIOLET LIGHT-INDUCED TUMORS. Toshiyuki Kitajima, Michihiro Iwashiro, Kagemasa Kuribayashi, Sadao Imamura, Departments of Dermatology, and Immunology and Cell Biology, Kyoto University, Kyoto, Japan.

To investigate the immunological characteristics of tumors arising in the idientical condition, we established ultraviolet light (UV)-induced tumors from (BALB/c x C57BL/6)F_1 mice, and analyzed effector T cell subsets involved in the tumor rejection. Among six lines of UV tumors used in this experiment, five were fibrosarcomas (♂1, ♀ 1, 5.2B, 2.1F, 6.1B) and one was endothelial tumor (♀ 2). All tumors grew progressively in nude mice, but were rejected in syngeneic normal mice. This rejection was blocked by *in vivo* administration of anti-CD8 mAb, but not by anti-CD4 mAb, indicating the presence of antitumor immune response mediated by $CD8^+$ T cells. Cytotoxic activities of MLTC cells against each of these tumors revealed that restriction molecules were all of $H\text{-}2^d$ origin, that is K^d, D^d, and L^d MHC class I, suggesting the presence of some preferential mechanisms. In addition, antigen-loss variant R95C derived from ♂1 by treatment with specific CTL clones was also rejected in syngeneic mice. In contrast to the parent line, the rejection of R95C was blocked by administration of either anti-CD4 or anti-CD8 mAb. R95C-specific CTL was also induced and had crossreactivity to ♂1. Moreover, UV-irradiated mice rejected the parent tumor, but not R95C. Nude mice reconstituted with a combination of $CD4^+$ T cells from UV-irradiated mice and $CD8^+$ T cells from normal mice did not reject R95C, suggesting some functional defects of $CD4^+$ T cells in UV-irradiated mice against R95C. These results indicate that UV tumors provide useful system for analyzing individual tumor antigens.

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PEMPHIGUS VULGARIS ANTIGEN (PVA) LACKS CLASSICAL BIOCHEMICAL PROPERTIES OF CADHERINS. R. Todd Plott, Masayuki Amagai, Vera Klaus-Kovtun and John R. Stanley, Dermatology Branch, NIH, Bethesda, MD, USA

The extracellular (EC) domain structures of PVA and classical cadherins (CCs) (e.g. E-cadherin [Ecad]) are similar and share about 50% amino acid homology as well as consensus calcium binding sites. PVA and CCs also share an intracellular (IC) segment with about 40% homology. PVA is, therefore, a member of the cadherin supergene family. CCs have certain well-defined biochemical properties: the EC region is resistant to degradation by 0.01% trypsin in 1 mM calcium (T/C); cultured cells shed a T/C-resistant 80 kD fragment of the EC domain; the IC domain binds to α, β, and γ catenins. To test these properties for PVA we used keratinocytes that display both PVA and Ecad. Whereas keratinocytes shed the 80 kD, T/C-resistant Ecad fragment into the media, as detected by immunoprecipitation (IP) with an anti-Ecad Mab, PV sera did not precipitate any fragments from the media. After T/C treatment, IP showed Ecad remained cell-associated and intact, but PVA was cleaved to a stable 35 kD fragment, precipitated by PV sera from the T/C medium. Cleavage of this specific fragment is not a result of the IC domain changing the conformation of the EC domain, because the same fragment was obtained by T/C treatment of L cells transfected with cDNA producing a chimeric molecule with the PVA EC domain and the Ecad IC domain. Flow cytometry confirmed loss of PVA from the L cell surface. IP of Ecad from keratinocytes demonstrated co-precipitation of the catenins, whereas only plakoglobin was co-precipitated with PVA. Plakoglobin co-migrated with γ-catenin, suggesting that these are the same molecule. Therefore, although in the cadherin gene superfamily, PVA does not share many of the classical properties of cadherins.

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CADHERIN DEPENDENT REGULATORY MECHANISMS OF VASCULAR ENDOTHELIAL CELL-CELL INTERACTIONS. Norihisa Matsuyoshi, Ken-ichi Toda, Yuji Horiguchi, Toshihiro Tanaka and Sadao Imamura, Department of Dermatology, Kyoto University Faculty of Medicine, Kyoto, Japan

Cadherins are calcium dependent cell adhesion molecules, playing a key role in cell-cell interactions of various kinds of cells. However the properties of vascular endothelial cell (VEC) cadherin are not well known. To learn more about the molecules, we established the monoclonal antibody (ENCD1) which specifically inhibit the cell-cell adhesion of VEC, by immunization of murine endothelial cell line (F2) treated with trypsin containing 1mM Ca^{++}(TC). ENCD1 had following characters. (1) It caused the cell-cell detachment of endothelial cells. (2) Immunocytochemistry demonstrated that cell-cell borders of F2 cells were specifically stained, whereas those of other cell types were not. (3) Immunoblot analysis showed the single band with a molecular weight of 130Kd. This protein band was preserved by TC treatment, but not by TE (trypsin with 1mM EGTA) treatment. These data clearly demonstrated that this antigen was VEC specific cadherin. To clarify its function, the cell-cell adhesiveness and the cell barrier properties were measured. A co-cultivation of ENCD1 with F2 cells induced the cellular dissociation in cell aggregation assay and destroyed the honeycomb structure on Matri-gel. Furthermore ENCD1 disrupted the barrier properties in DAB permeability assay. These observations indicate that VEC specific cadherin plays a crucial role in cellular integrity and permeability.

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CADHERINS MEDIATE HUMAN MELANOCYTE ADHESION TO KERATINOCYTES IN VITRO. Aimin Tang, Mark S. Eller, Masahiro Hara, Mina Yaar and Barbara A. Gilchrest, Department of Dermatology, Boston University School of Medicine, Boston, MA

Cadherins are a family of transmembrane glycoproteins that mediate calcium-dependent cell-cell adhesion. Two types have been identified in human epidermis: E-cadherin (E-cad) present in all the viable layers and P-cadherin (P-cad) restricted to the basal layer. Recent studies suggest that E-cad mediates keratinocyte (Kc)-Kc and Langerhans cell-Kc interactions. To determine whether cadherins are also involved in melanocyte (Mc)-Kc interactions we performed flow cytometry analysis with the monoclonal antibody(mAb), DECMA-1, to E-cad. Cultured human Mc strongly expressed E-cad comparable to its level of expression on Kc. Treatment with 0.01% trypsin in 1mM EDTA (TE) decreased the level of E-cad expression on Mc and Kc by ~ 95% as compared to treatment with 0.01% trypsin in 1mM calcium (TC) which did not alter its level of expression. Northern blot analysis of total RNA from Mc and Kc hybridized with human E-cad and P-cad cDNAs detected the reported ~4.5 kb and ~3.2 kb transcripts respectively. To assess the adhesion of Mc to Kc, Mc were added to a Kc monolayer and after 1h the cultures were rinsed to remove unbound cells; total melanin content of the remaining cells was measured. Ninety-seven percent of Mc adhered to Kc at 37°C as compared with only ~2% at 4°C, consistent with temperature dependent requirement for adhesion molecule clustering. TE treatment of Mc decreased their adhesion to Kc by ~80% as compared to TC treatment, consistent with the known ability of calcium to protect cadherin degradation by trypsin. Adhesion of Mc to Kc was not inhibited by mAbs to several human β1 integrins but was partially (27%) inhibited by anti-E-cad antibody suggesting a role for an additional adhesion molecule, probably P-cad in mediating Mc-Kc interaction. Adhesion of Mc to Fb was comparable to Kc, but anti-E-cad could not inhibit adhesion of Mc to Fb establishing that E-cad mediates Mc binding only to Kc but not to Fb. We conclude that human Mc express E-cad and P-cad, and that Mc-Kc binding is mediated at least in part by functional E-cad. Our data suggest an important role for cadherins in the epidermal-melanin unit.

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DISTRIBUTION AND CHARACTERIZATION OF E-CADHERIN EXPRESSED BY MURINE DENDRITIC CELLS. BJ Van Dyke, TA Borkowski, K Schwarzenberger, VW McFarland and MC Udey, Dermatology Branch, NCI, Bethesda, MD, USA

Recent studies indicate that murine Langerhans cells (LC) synthesize and express E-cadherin (E-cad) that mediates adhesion of LC to normal keratincytes in vitro. To determine if E-cad is selectively expressed by LC as compared with other dendritic cells (DC), we isolated DC from various tissues and assessed expression of E-cad and DC surface antigens by flow cytometry. DC from BALB/c spleen and thymus were released with collagenase, floated on 35% BSA gradients and additionally enriched via transient adherence to plastic. DC from inguinal and axillary (skin-associated) or mesenteric (gut-associated) lymph nodes (LN) were released with collagenase, and floated on 14.5% metrizamide gradients. Cells were analyzed after staining with anti-E-cad mAb (or mAb reactive with DC) and appropriate secondary Ab. Preparations enriched in splenic DC and DC from gut-associated LN were devoid of E-cad expressing cells. Some DC from thymus and skin-associated LN appeared to express low levels of E-cad, however. We also cultured blood DC from mice treated with cyclophosphamide (200 mg/kg IP) 8 days before phlebotomy. After 10 days in media supplemented with GM-CSF (10 ng/ml), we recovered DC functionally and phenotypically identical to those described by Inaba and coworkers (J. Exp. Med. 175:1157, 1992). Blood DC expressed immunoreactive E-cad in addition to DC surface antigens. We isolated blood DC RNA and detected mRNA encoding portions of both extracellular and intracellular regions of E-cad using E-cad specific primers and RT-PCR. E-cad immunoprecipitated from blood DC also comigrated with that from fibroblasts transfected with E-cad cDNA. These results indicate that among DC, E-cad is selectively expressed by LC, cells that may give rise to LC (blood DC), cells that may be derived from LC (skin-associated LN DC) and cells that may be closely related to LC (thymic DC). These studies also confirm that E-cad expressed by murine DC is identical to that expressed by other cells.

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THE EXTRACELLULAR DOMAIN (EC) OF PEMPHIGUS VULGARIS ANTIGEN (PVA) MEDIATES WEAK HOMOPHILIC ADHESION. Masayuki Amagai, Sarolta Karpati, Vera Klaus-Kovtun, Mark C. Udey, John R. Stanley, Dermatology Branch, NIH, Bethesda, MD, USA

PVA is in the supergene family of cadherins, transmembrane proteins that mediate calcium-dependent homophilic cell adhesion. Initially described cadherins, such as E-cadherin (Ecad), mediate adhesion when transfected into mouse fibroblasts (L cells) only if their intracellular region (IC) interacts with the cytoskeleton through binding to α, β, and γ catenins, which are present in L cells. This cytoskeletal interaction is thought to cluster the molecules in the membrane, thereby increasing cadherin avidity. To determine if the PVA EC mediates adhesion and to compare it to the Ecad EC, we transfected L cells with Ecad cDNA or with a chimeric cDNA encoding the PVA EC and the Ecad IC (PVCad). Both encoded proteins were expressed on the cell surface, as assessed by flow cytometry with anti-Ecad EC monoclonal antibody and PV sera. The IC of both molecules bound the catenins equally as shown by their co-immuno-precipitation with Ecad and PVCad. When single cell suspensions, obtained by EDTA, were allowed to aggregate in 1 mM calcium, the Ecad cells showed strong aggregation with large clumps, whereas the PVCad cells formed small aggregates that could be easily disrupted. However, the PVCad aggregation was specific and homophilic because when PVCad cells were mixed with control L cells transfected with the neomycin-resistance gene, and one population was labeled with a fluorescent cytoplasmic dye, only PVCad cells were in the aggregates. We conclude that the PV EC functions weakly in homophilic adhesion, and speculate that the cytoplasmic factors promoting adhesion may be different for Ecad and PVA.

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ROLE OF INTEGRINS IN HUMAN MELANOCYTE ATTACHMENT AND DENDRICITY. Masahiro Hara, Mina Yaar, Aimin Tang, Wende R. Reenstra, Mark S. Eller and Barbara A. Gilchrest, Department of Dermatology, Boston University School of Medicine, Boston, MA

Integrins mediate cell attachment to extracellular matrix (ECM) in the presence of divalent cations. To identify those mediating human melanocyte interaction with ECM, cells were plated in serum-free cation-containing medium on dishes coated with 3μg/cm² of fibronectin (FN), laminin (LM), type IV collagen (C IV), vitronectin (VT), type I collagen (C I) or bovine serum albumin (BSA). Prominent dendricity was seen in cells on LM, C IV and C I as compared to polygonal spreading without dendrites in cells on FN and VT. Attachment rates were high and comparable on all ECM. Cells on BSA did not attach. Anti-integrin antibody binding on disaggregated cells analysed by flow cytometry showed strong and comparable binding of all antibodies studied (α2,α3,α5,αV and β1), but confocal image analysis of plated cells suggested that the ECM strongly influenced integrin cell surface distribution. In order to assess the role of individual cations in integrin-mediated cell attachment, cells were plated on ECM in specially formulated medium in the presence or absence of 1.0 mM calcium (Ca), magnesium (Mg), or manganese (Mn) or combinations thereof. The best attachment to all ECM was observed with Mn (2 to 5 fold more than with Ca or Mg). Addition of Ca and/or Mg to Mn did not further enhance attachment, suggesting a major role for Mn in integrin mediated attachment. In the presence of all 3 cations, cells in addition were highly dendritic on LM≅C IV>C I but omission of any one cation resulted in polygonal morphology. Preincubation of cells with RGD, a known attachment peptide for integrins, decreased attachment to LM and C IV by approximately 60-70% and to FN, VT, or C I by 97-98% . Our data confirm that melanocytes express a wide variety of integrins that are critical for their attachment to ECM. We further demonstrate that integrins require Mn to mediate ECM attachment and Ca, Mg, and Mn in combination to mediate dendricity.

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TRANSGENIC BASAL KERATINOCYTE-SPECIFIC EXPRESSION OF ICAM-1 IN MURINE SKIN. Ifor Williams and Thomas S. Kupper. Division of Dermatology, Harvard Medical School/ Brigham and Women's Hospital, Boston MA.

Expression of ICAM-1 by keratinocytes in lesions of inflammatory skin disease is invariably associated with leukocyte (often T lymphocyte) infiltration of skin. In certain cases, keratinocyte ICAM-1 expression precedes lymphocyte infiltration of skin, leading to speculation that epidermal expression ICAM-1 may serve to initiate leukocyte-mediated inflammatory skin disease. To test this hypothesis, the murine ICAM-1 cDNA was incorporated into an expression vector containing 2.0 kb of the 5' flanking sequence of the human K14 gene. This construct, previously shown to contain elements that direct transgene expression to basal keratinocytes, was microinjected into fertilized eggs of FVB/N mice. Five founder mice were identified by PCR as well as Southern blot analysis. Lines derived from these founder mice showed ICAM-1 mRNA expression in the predicted tissue specific pattern, and immunohistochemical analysis showed cell surface ICAM-1 expression on basal keratinocytes. Despite high level constitutive ICAM-1 expression in epidermis, no evidence for spontaneous inflammatory cell infiltration of skin was observed. However, after perturbation of K14/ICAM-1 mouse skin, certain patterns emerged that were seen exclusively in transgenics and not in non-transgenic littermate controls. These included significant epidermal hyperplasia after syngeneic skin grafting and exaggerated cytotoxic killing of follicular epithelial keratinocytes after local injection of activated alloantigenic T cells. It is concluded that ICAM-1 expression by basal keratinocytes, as an isolated event, does not lead to cutaneous inflammation. However, unregulated expression of keratinocyte ICAM-1 can influence the nature and duration of certain kinds of elicited inflammation in skin.

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DESMOYOKIN, A 680 KD KERATINOCYTE PLASMA MEMBRANE-ASSOCIATED PROTEIN, IS HOMOLOGOUS TO THE PROTEIN ENCODED BY HUMAN GENE AHNAK: FURTHER STUDIES FOR THE MECHANISM OF ITS TRANSLOCATION TO THE PLASMA MEMBRANE. Takashi Hashimoto,[1] Nobuyoshi Shimizu,[2] Yasuo Kitajima,[3] and Takeji Nishikawa,[1] Departments of [1]Dermatology and [2]Molecular Biology, Keio University School of Medicine, Tokyo, and [3]Department of Dermatology, Jichi Medical College, Tochigi, Japan

We have cloned a mouse cDNA (DY6;3693bp) which encodes desmoyokin, a 680 kD desmosomal plaque protein. DY6 consists of highly homologous repeats about 128 residues long. Surprisingly, the amino acid sequence showed a significant homology with AHNAK, a newly identified human gene encoding a 700 kD protein which was suggested to be downregulated in neuroblastoma. From its extensive homology, the similarity in both size and structure and the identical patterns on Southern blot analysis of genomic DNAs, desmoyokin and AHNAK protein are thouhgt to be an identical protein. Although the desmoyokin/AHNAK protein is detected in a variety of cell types for both protein and mRNA levels, its distribution in keratinocytes (associated closely with cell membrane) is quite different from that in all cells other than keratinocytes (distributed diffusely in the cytoplasm). These findings suggest that the desmoyokin/AHNAK protein is a ubiquitous molecule with a unique structure and has different distributions (and probably different functions) among different cells. Because the desmosome is known to be regulated by Ca^{2+} in the culture medium, we also examined the effect of the extracellular Ca^{2+} in the distribution of desmoyokin/AHNAK protein in keratinocytes. In various keratinocyte cell lines, by shifting Ca^{2+} concentration from low to high, the cytoplasmic pool of the desmoyokin/AHNAK protein was translocated to the plasma membrane in the similar pattern to desmoplakin. This protein is phosphoprotein, and, therefore, protein kinase C may play a role in this phenomenon.

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CHARACTERIZATION OF KERATIN K6 GENE EXPRESSION USING TRANSGENIC MICE.
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Keratin K6 expression is determined by the state of development, differentiation and hyperproliferation of the keratinocyte. In normal epidermis K6 expression is limited to the hair follicle but can be induced in the interfollicular epidermis by conditions that perturb normal keratinocyte biology. We have isolated two K6 genes from the mouse genome and found them to be linked, similarly orientated and transcriptionally active. Sequence analysis has revealed that although the coding regions are remarkably similar, with both genes essentially encoding the same protein, their respective promoter regions are distinctly different. This suggests that the two K6 genes would be differentially expressed. In an attempt to define the expression characteristics of the K6 genes, we have produced transgenic mice with a 13.5 Kbp fragment encoding one of the mouse K6 genes. This fragment contains a complete K6 gene with 7.7 kbp of 5' flanking and 1.5 kbp of 3' flanking sequences. The c-terminal of the K6 protein was replaced with the c-terminal epitope of human K1 to enable co-analysis, by double-label immunofluorescence, of expression of both the K6 transgene and the endogenous K6 genes. Expression during development was first observed at day 15 in the periderm and paralleled that of endogenous K6. The K6 transgene was expressed, post-natally, in the same differentiation-compartment of the hair follicle as the endogenous genes. The transgene was expressed in the cheek pouch, tongue and esophagus but not in the stomach, bladder, liver or spleen. However, differential expression of the transgene was observed in the tongue with expression limited to the papillae while endogenous K6 was expressed throughout the tongue epithelium. The topical application of retinoic acid or TPA (12-0-tetradecanoylphorbol-13-acetate) induced expression of the transgene in the non-follicular epidermis of these mice. These studies have established that the 13.5 kbp transgene contains all the cis regulatory elements required for correct tissue-specific expression of K6. Studies are now underway to determine whether differential expression is a result of the transgene lacking certain cis elements or whether faithful reproduction of endogenous expression requires transcription of both K6 genes.

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TISSUE-, DIFFERENTIATION- AND DEVELOPMENTAL-SPECIFIC EXPRESSION OF THE HUMAN LORICRIN GENE IN TRANSGENIC MICE.
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The cornified cell envelope (CE) of terminally differentiated epidermis is a complex structure consisting of several defined proteins of which loricrin is the major component. Human loricrin has a molecular weight of 26kDa, and consists of three long glycine-serine-cysteine rich sequence domains that contain quasi-repeating peptides which form the novel glycine loop motif. We have recently characterized genomic DNA fragments that contain the full-length loricrin gene, which has a simple structure with a single intron in the 5'-untranslated region. In order to further study the expression, structure and function of loricrin, we have produced transgenic mice using a 20 kb fragment containing the human loricrin gene. To discriminate between expression of the human loricrin and the endogenous mouse loricrin, we tagged the C-terminal end of human loricrin with the substance P neuropeptide. RNA-PCR indicates that the human loricrin gene is expressed tissue specifically, that is, expressed in epidermis, palate, tongue and stomach, and not expressed in brain, heart, kidney, lung, skeletal muscle, and spleen. In addition, developmental studies indicate that both the human and endogenous mouse loricrin genes are induced at day 16 in the skin. Indirect immunofluorescence analyses using double labeling reveal that the distribution of human loricrin is identical to mouse loricrin. Immunogold studies show the expression of the human loricrin in L- and F-keratohyalin granules of the epidermis. At both the mRNA and protein levels, human loricrin is expressed about 125% of the level of mouse loricrin; that is, the transgenic mouse epidermis contains 2-3 times more loricrin than the non transgenic mouse epidermis. These data suggest: 1) the trans-acting factors which control the mouse gene system are sufficient to permit the proper expression of the human loricrin gene. 2) Our 20kb construct contains most if not all of the regulatory sequence elements required for correct tissue, temporal, and developmental-specific expression. 3) Significant overexpression of human loricrin presumably produces much thicker CE, which does not cause any pathological skin changes.

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DELETION SUBCLONING REVEALS SOPHISTICATED CELLULAR REGULATION OF THE TRANSGLUTAMINASE1 ENZYME.
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The formation of the cornified cell envelope in terminally differentiating epidermis involves three different transglutaminases (TGases), the membrane-associated TGase1, ubiquitous tissue TGase2 and proenzyme TGase3. Although information is available on their sequences, properties and expression, little is known about their precise role(s). We have made a series of deletion constructs of the TGase1 system from our full-length cDNA. Following expression in bacteria and purification, the forms were characterized with respect to their specific activties and substrate specificities. Deletion of the first 30-63 amino acids resulted in a ten-fold increase in specific activity. Their activity could be further increased two-fold on dispase treatment. Deletions beyond residue 63 resulted in a less dramatic change, and deletions beyond 109 residues lost activity. Similarly, deletions of the last 240 residues retained activity, but deletions beyond this lost activity. Thus we can define the minimal sequences required for activity. The data indicate TGase1 has been modified and enlarged during evolution by addition of sequences that effectively brake its specific activity and mask proteolytic activation sites common to other TGases. Constructs in which the first 62-97 had been deleted showed dramatic variations in substrate specificities toward synthetic peptides corresponding to sequences of putative TGase substrates. Indirect immunofluorescence experiments using a new antibody to the smallest active construct defined above decorated all levels of the epidermis, with some potentiation in the granular layer. By Western blotting we found that boiled SDS extracts of cultured HaCat or RHEK cells contain a series of smaller processed TGase1 forms which also possess high specific activities. The presence of such forms in living cells suggests that epithelial cells can regulate both the specific activity and substrate specificity of the TGase1 system by controlled proteolytic processing during terminal differentiation.

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MOUSE 230-kDa BULLOUS PEMPHIGOID ANTIGEN GENE: CHARACTERIZATION OF 5'-FLANKING REGION.
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230-kDa bullous pemphigoid antigen (BPAG1) is the major autoantigen in bullous pemphigoid. To clone the 5'-flanking region of mouse BPAG1 gene, we screened a mouse genomic library with a human 0.3 kb cDNA and isolated a ca. 15 kb clone. Southern hybridization allowed isolation of a 3 kb Hind III fragment which was subject to nucleotide sequencing. Alignment of the mouse nucleotide sequences with human BPAG1 sequences revealed that the 3.0 fragment contained 2.4 kb of 5'-flanking region, addition to exon 1 and exon 2. TATAAA and CCAAT consensus sequences, as well as several putative cis-regulatory sequences were identified in the 5'-flanking region. The functionality of the promoter region was demonstrated by development of promoter/CAT reporter gene constructs, followed by transfections of normal mouse keratinocytes and PAM cells in culture. The CAT assays revealed significant promoter activity in both types of cells. Also transfection study using PAM, NIH3T3, B16 melanoma, and CT26 colon carcinoma cells suggests that 1.1 kb 5'-flanking DNA contains cis-acting elements which confer tissue specificity to the expression of BPAG1 gene.

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THE GENE EXPRESSIONS OF TYROSINASE AND TYROSINASE-RELATED PROTEIN ARE ENHANCED BY DIFFERENT *CIS*-ACTING ELEMENTS.
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Tyrosinase is a rate-limiting enzyme of melanin biosynthesis. Tyrosinase-related protein (TRP, brown-locus protein) is involved in the production of black melanin rather than brown. Both tyrosinase and TRP share a significant homology (about 40%) in amino acid sequence, are specifically expressed in melanocytes, are induced similarly by several biological factors, and are probably derived from a common ancestral gene. It is therefore important to know whether the gene expressions of both tyrosinase and TRP are regulated by a similar promoter function.

We found the *cis*-acting element, located between 2.0 and 1.8 kilobase pairs (Kbp) upstream from the transcription initiation site of human tyrosinase gene, that enhances the transient expression of the luciferase reporter gene in melanoma cells, but not in Hela cells. We further identified the pigment cell-specific enhancer of 39-bp core element between -2.0 and -1.8 Kbp responsible for the expression of the human tyrosinase gene. On the other hand, the downstream region of TRP gene, including the first intron, enhances the transient expression of the luciferase reporter gene under control of the TRP gene promoter. This enhancer-like activity is detected not only in melanoma cells but also in Hela cells whose TRP gene expression is assumed to be repressed. These results indicate that the promoter function of the human tyrosinase gene is completely different from that of the human TRP gene.

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JUN-B PROTO-ONCOGENE MEDIATES TRANSFORMING GROWTH FACTOR-β-INDUCED INHIBITION OF COLLAGENASE GENE EXPRESSION.
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We have investigated the molecular mechanisms by which TGF-β interacts with pro-inflammatory cytokines, interleukin-1 (IL-1α and IL-1β), and tumor necrosis factor-α (TNF-α), in modulating fibroblast collagenase gene expression *in vitro*. TGF-β blocks the induction of collagenase gene expression induced by the cytokines studied, as determined at the mRNA steady-state level and by transient cell transfections with a collagenase promoter/CAT reporter gene constructs. Also, TGF-β counteracts cytokine-induced *trans*-activation of a 3xTRE-thymidine kinase (tk) promoter/CAT gene construct. Analysis of the expression pattern of oncogenes of the Jun family revealed that the two forms of IL-1 and TNF-α promote high levels of *c-jun* mRNA, independently of the presence of TGF-β. By contrast, TGF-β has little effect on *c-jun* expression but is a potent inducer of *jun-B* mRNA, in the absence or in the presence of the other cytokines. Also, a transient elevation of *c-fos* mRNA levels was noted following fibroblast stimulation by any of the growth factors studied. Using expression vectors for *c-jun* and *Jun-B* in transient cell transfections, we show that *c-Jun trans*-activates both the collagenase promoter and the 3xTRE-tk promoter/CAT constructs in fibroblasts, and this effects is abrogated by *Jun-B*. Furthermore, overexpression of *Jun-B* prevents TNF-α-induced *trans*-activation of both promoters. Taken together, these data demonstrate that TGF-β counteracts cytokine-induced collagenase gene expression through the induction of high levels of expression of *Jun-B* proto-oncogene.

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IDENTIFICATION OF THE IFN-γ RESPONSIVE ELEMENT WITHIN THE HUMAN ICAM-1 GENE. N Shibagaki, L-J Li, S Naik, T Nguyen, SW Caughman, Department of Dermatology, Emory University School of Medicine, Atlanta, GA, USA.

The expression of ICAM-1 is rapidly and markedly induced in epithelial cells, such as keratinocytes, after exposure of IFN-γ. We have previously cloned and characterized the 5' flanking transcriptional regulatory region of this gene. Using CAT reporter gene constructs containing various portions of this gene, we identified the minimal basal promoter region (-88/-8) and the critical 5' limit for IFN-γ-responsiveness (-88) of the ICAM-1 gene. To further elucidate the specific response element (RE) of the ICAM-1 gene that is both necessary and sufficient for this IFN-γ effect, we have created and utilized an additional series of CAT reporter constructs driven by the thymidine kinase minimal promoter into which candidate ICAM-1 IFN-γ RE regions have been cloned. Using this approach, we have clearly identified that the minimal region required and sufficient for IFN-γ-dependent enhancement of transcription is located between base pairs -79 and -65. This regions contains two motifs, GTTTC and AAA, that are also found within the recently characterized IFN-γ-responsive region of the guanlylate binding protein gene. Utilizing gel mobility shift assays with various double stranded oligonucleotides spanning all or portions of this -79/-65 region, we detected specifc DNA-protein retardation effects using nuclear protein extracts isolated from IFN-γ-treated, but not untreated, human keratinocytes. A minimal 11 bp palindromic region, -76/-66, is capable of binding to the IFN-γ-induced protein, but 3' deletion to -69 detroys the DNA-protein interaction. These studies identify novel sequences involved in the regulation of the human ICAM-1 gene, and perhaps other genes, in response to the pro-inflammatory signals transduced by IFN-γ.

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IMMUNOLOGICAL AND FUNCTIONAL QUANTITATION OF NUCLEAR RETINOID X (RXR) AND RETINOIC ACID (RAR) RECEPTOR PROTEINS IN HUMAN EPIDERMIS *IN VIVO*: HIGH LEVELS OF RXRS AND RAR-γ, LOW LEVELS OF RAR-α, AND ABSENCE OF RAR-β GJ Fisher, HS Talwar, J-H Xiao, SC Datta, AP Reddy, M-P Gaub, C Egly, P Chambon, JJ Voorhees, Department of Dermatology, University of Michigan, Ann Arbor, MI, USA, LGME/CNRS, U184/INSERM, Faculte de Medecine, Strasbourg, FRANCE.

All-*trans* and 9-*cis* retinoic acid (RA) regulate gene expression via two distinct families of nuclear receptors, RAR and RXR. RARs are activated by both all-*trans* and 9-*cis* RA, while RXRs are activated by 9-*cis* RA, but not all-*trans* RA. RAR and RXR families are each encoded by three genes designated alpha (α), beta (β), and gamma (γ). RARs and RXRs function as homo- and heterodimers. Biological responses to retinoids are dependent on the types and levels of individual RARs and RXRs expressed in target tissues. Therefore, we have determined the ratio of all RXRs to all RARs, and the levels of RAR-α, β, and γ protein, in nuclear extracts from human epidermis. The total numbers of all RXRs and all RARs, measured by ligand binding assays, were 10150±1035 (n=4) and 1670±115 (n=12) receptors per cell, respectively, a 6 to 1 ratio of RXR to RAR. To assess their relative levels, RAR-α, β, and γ were each specifically depleted by immunoprecipitation, and the amount of remaining non-precipitated receptors determined by ligand binding. RAR-γ was most abundant, representing 90±11% (1510±405 receptors/cell, n=6) of total RARs, while RAR-α, represented 10±1.5% (160±25 receptors/cell, n=6). No RAR-β was detected (n=5). Similar results were obtained by direct quantitation of immunoprecipitated receptors by Western analysis, using known amounts of recombinant receptors as standards: RAR-α, 12±3% (170±15 receptors/cell n=5); RAR-β, not detected (n=4); RAR-γ 88±12% (1215±151 receptors/cell, n=5). Nuclear extracts from human epidermis formed a specific complex with the Retinoic Acid Response Element (RARE) from the RAR-β2 gene. Supershift gel retardation with antibodies to RAR-α, β and γ detected RARE-RAR-γ and RARE-RAR-α complexes in a ratio of 4 to 1. No RARE-RAR-β complex was detected. RARE-RAR-γ-complexes were completely shifted (double supershifted) by antibody to RXR, indicating that all RAR-γ bound to RARE as RXR/RAR-γ heterodimers. These data identify RAR-γ/RXR heterodimers as the major transcription factors mediating gene transcription by all-trans and 9-cis RA in human skin *in vivo*.

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FROM JOSEPH PLENCK (1735-1807) TO KEIZO DOHI (1866-1931) AND TODAY. AUSTRIAN INFLUENCE ON DERMATOLOGY IN JAPAN. Karl Holubar, Bettina Kokert, Cathrin Schmidt, Departments of Dermatology and the History of Medicine, University of Vienna, Vienna, Austria

Modern medicine developed in Vienna as an offspring of the Leiden School in the 2nd half of the 18th century. Through this Dutch connection works of Gerard van Swieten (1700-1772) or Anton Stoerck (1731-1803) reached Japan as much as the writings of Joseph Plenck, author of the"Doctrina de morbis cutaneis",1776 and "Doctrina de morbis venereis", 1778.

One century later Keizo Dohi wrote, that Plenck's oeuvre will remain unforgotten in the history of dermatology, for his importance, in general and for the close relation to the development of medicine and dermatology in Japan. A search for Japanese translations of Plenck's books could prove that. Dohi himself wanted to become a surgeon but changed his plans and enrolled in Moriz Kaposi's (1837-1902) department in Vienna as a postgraduate student (1893). Sifting through Dohi's textbook and papers, the importance of the Hebra - Kaposi School for Japan is explicitely stated by the Japanese master, who founded the Japanese Dermatological Society in December of 1900 and the disciplines's journal in 1901. The language of publication was German. Dohi's first paper appeared in the Archiv für Dermatologie und Syphilis in 1896, in German.

In recent years Austrian dermatology has become influential again in dermatological research after a mid-century low. A series of investigators have spent sabbatical years or postgraduate training at the former Hebra - Kaposi Department in Vienna. Their scientific achievements were mainly in the field of immunobiology of the skin. A list of salient publications and their authors is presented.

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FULL SIZED AND FUNCTIONAL THROMBOMODULIN EXPRESSION ON NORMAL AND NEOPLASTIC KERATINOCYTES AS A NOVEL DIFFERENTIATION MARKER OF KERATINOCYTES. Hitoshi Mizutani, *Tatsuya Hayashi, Shin Inachi, Satoshi Ohyanagi, *Kouji Suzuki, **Kouji Hashimoto, Masayuki Shimizu, Dep.Dermatol, *Dep.Mol.Biol.Gen.Dis., Mie University Faculty of Medicine, Tsu, **Dep.Dermatol., Osaka Univ., Japan

Thrombomodulin (TM) is a protein with strong anticoagulation properties that acts at the level of endothelium and is a human isoform of mouse development antigen; fetomodulin. TM gene expression is known to be modulated by proinflammatory cytokines in endothelial cells. In order to better understand the tissue distribution of TM, we used immunohistochemistry to examine normal and neoplastic skin biopsies. Significant TM protein was observed on suprabasilar keratinocytes, while basal and terminally differentiated keratinocytes were negative. Both seborrheic verruca and squamous cell carcinoma were positive. Extracts of skin and SV 40 transformed keratinocytes showed immunoreactive TM protein that appeared identical in molecular weight and relative abundance to TM derived from endothelial cells. Keratinocytes grown under low calcium conditions did not express TM. When TM derived from keratinocytes was compared to endothelial TM, binding to thrombin and cofactor activity were similar, while regulation in response to stimuli (e.g., IL-1, TNF-alpha) was different. Thus, epidermal TM appears similar or identical to endothelial TM, and is furthermore a novel differentiation related membrane protein in keratinocytes. Paracrine roles of TM in epidermis independent of effects on coagulation remain to be explored.

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GANGLIOSIDE-INDUCED TERMINAL DIFFERENTIATION AND BIPHASIC 1,2-DIACYLGLYCEROL PRODUCTION IN HUMAN KERATINOCYTES. Mariko Seishima, Yumi Aoyama, Shunji Mori and Yoshinori Nozawa*, Departments of Dermatology and *Biochemistry, Gifu University School of Medicine, Gifu, Japan

Prior studies have indicated that gangliosides may be important regulators of cellular differentiation in keratinocytes. We examined the signal transduction in cultured human keratinocytes treated with 25 μg/ml GQ_1b tetrasialoganglioside containing two disialosyl residues. The mass contents of 1,2-diacylglycerol (DG) and inositol 1,4,5-trisphosphate (IP_3) were measured for keratinocytes exposed to GQ_1b. GQ_1b induced a biphasic DG increase in human keratinocytes. The first prominent peak was coincident with the increase in IP_3 derived from phosphatidylinositol-4,5-bisphosphate, while the second sustained phase of DG might be partly derived from phosphatidylcholine hydrolysis via phospholipase D (PLD) because DG formation was reduced by 30% in the cells treated with 0.5% ethanol. Furthermore, the involvement of PLD in DG production was confirmed by phosphatidyl-ethanol (PEt) formation in 0.5% ethanol-pretreated cells. These findings suggest that PLD activation might be involved in the terminal differentiation induced by GQ_1b in human keratinocytes.

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MONOCLONAL ANTIBODY (7C1) RELATED TO LAMELLAR GRANULES AND INTERCELLULAR MATERIAL MAY BE A RELIABLE MARKER FOR CELL DIFFERENTIATION OF KERATINIZING TUMOR CELLS. Hiroyuki Suzuki, Mami Murase, Ryoko Miyamoto and Shunichi Baba, Department of Dermatology, Surugadai Nihon University Hospital, Tokyo, Japan

We have generated a monoclonal antibody, 7C1, from an extract of pig snout skin. Immunohistochemistry (IHC) revealed that the 7C1 antigen is specific to the epidermis. Western blots revealed the 7C1 antigen to be a protein with a molecular weight of 210 kD. To clarify 1) the subcellular localization of 7C1 antigen in normal human epidermis, and 2) the clinical utility of 7C1 in cutaneous keratinizing tumors, we performed the present study using IHC and immunoelectron microscopy (IEM). Normal human epidermis was examined with 7C1 using immunogold techniques on ultrathin cryosections. Various keratinizing tumors, such as SCC, BCC and inverted follicular keratosis, were tested with 7C1 and anti-PCNA antibody using the ABC method. IEM localized the 7C1 antigen to the lamellar granules in the keratinocytes and the intercellular spaces in the upper layer of the normal human epidermis. In the tumors examined, 7C1 antigen was clearly seen in squamoid granular cells, but not detected in basaloid cells. PCNA was distributed mainly in basaloid cells. These results suggest that 1) the 7C1 antigen is derived from the lamellar granules in the epidermal keratinocytes and fills the intercellular spaces of the epidermis, and that 2) 7C1 may be useful as a reliable marker for cell differentiation of cutaneous keratinizing tumor cells.

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EXPRESSION OF TWO COLLAGEN BINDING STRESS PROTEINS, HSP47 AND OSTEONECTIN, IN KERATINOCYTES. Hitoshi Kudo, and Sadao Imamura, Department of Dermatology, Kyoto University Faculty of Medicine, Kyoto, Japan

HSP47 and osteonectin are both collagen-binding proteins expressed only in collagen-producing cells. They are concurrently inducible with type IV collagen through the differentiation of embryonic F9 cells. HSP47 is literally stress-inducible and osteonectin was also reported to be stress-inducible in some conditions. We have found that they are stress-inducible in keratinocyte-derived cell lines with Northern blot analysis and immunoprecipitation. They are also found to be expressed in normal human keratinocytes. Their control of expression during stress, growth, and differentiation are now under investigation. Their possible roles in collagen metabolism and wound healing of keratinocytes will be discussed.

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AMILORIDE BLOCKS THE NON-SPECIFIC CATION CHANNEL IN BASAL KERATINOCYTES. Theodora Mauro, Pamela Pappone. Dermatology Service, VAMC and Department of Physiology, University of California, Davis, Davis, California.

Previous investigations of keratinocytes have demonstrated a non-specific cation channel which is activated by, and permeant to, calcium. Amiloride has reported to block non-specific cation channels in other cells. In normal human keratinocytes, amiloride 10^{-6} M decreased current carried through the non-specific cation channel. Application of amiloride also decreased the Cl^- current of keratinocytes, probably by decreasing the Ca^{++} influx through the non-specific cation channel. Amiloride decreased the Ca^{++}-induced differentiation of keratinocytes, as measured by involucrin and cornified envelopes, in a dose-dependent fashion.

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KINETIC STUDIES AND DOSE-DEPENDENCY OF PORPHYRINSYTHESIS INDUCED BY EXOGENOUS 5-AMINOLEVULINIC-ACID IN CULTURED EPIDERMAL CELLS. Kurt-H. Jung[1], Bernd Thiele[1], Daniela Mayer[1], Helmut H. Wolff[2], Institute of Clinical Chemistry[1], Department of Dermatology and Venereology[2], Medical University of Lübeck, Germany

Porphyrin metabolism in liver cells in vitro following exposure to 5-aminolevulinic-acid (5-ALA) has extensively been investigated. Recently, it has been demonstrated that after application of exogenous 5-ALA to skin tumours, these tumour cells are capable to synthesize the photosensitizing protoporphyrin IX. Subsequent exposure to photo-activating light selectively destroys these tumours. However, porphyrinogenesis in keratinocytes, representing the decisive condition for the application of photodynamic therapy in epithelial tumours has not yet been studied.

In our preliminary studies we investigated the timecourse of porphyrin synthesis and the porphyrin pattern induced by exogenous applied 5-ALA in both an epidermal cell line (HaCaT) and human transformed cells (SCL II). (Cells were a kindly gift from P. Boukamp, DKFZ, Heidelberg).

Porphyrins were separated on a reversed phase column (C_{18}) following $HClO_4$-CH_3OH extraction and monitored by fluorescence spectroscopy. HaCaT as well as SCLII cells were cultured under standard conditions (DMEM, supplemented with aminoacids and vitamins, 10% FCS, 5% CO_2). For kinetic experiments confluent monolayers of cells (10^7 cells / 75 cm^2) were incubated with 5-ALA (1mM) in Hanks's solution for incubation periods from 20 minutes up to 8 hours. Dose dependency was assayed in HacaT cells with incubations periods of 2 hours. 5-ALA was added in increasing amounts to final concentrations ranging from 10 µM to 1 mM.

In summary, we found a rapid onset of porphyrinogenesis after incubation with 5-ALA. Protoporphyrin IX as the predominant product, as well as uroporphyrin III, heptaporphyrin, coproporhyrin III, and intermediate products were synthesized in a dose dependent manner in both cell types.

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REGULATION OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN RELEASE FROM NORMAL HUMAN KERATINOCYTES V. Morhenn, N. Sasaki, R.G. Rosenfeld, E.K. Neely. Department of Pediatrics, Stanford University, Stanford, CA 94305

Normal adult human keratinocytes (KC) grown in monolayer culture proliferate in response to insulin-like growth factors (IGF-I and IGF-II) or high concentrations of insulin, a mitogenic action thought to be mediated via the type 1 IGF receptor. The presence and regulation of IGF binding proteins (IGFBP-1 to -6), which modulate the delivery of IGF to the cell surface receptors, have not been investigated in this *in vitro* system. Conditioned media (CM) from confluent second to fourth passage KC maintained for 48 hr in KBM without added growth factors exhibited IGFBP bands at 42 and 38 kD (IGFBP-3), 33 kD, 28 kD, and 24 kD (IGFBP-4) in Western ligand blot (WLB), which utilizes SDS-PAGE, transfer to nitrocellulose, and overnight incubation with [^{125}I]-IGF-I or -II. Levels of IGFBP-3 were decreased approximately 80% in the presence of low concentrations of IGF-I or IGF-II (2-20 ng/ml) and high levels of insulin (1-10 µg/ml). Epidermal growth factor (EGF 0.1 ng/ml) resulted in a 60% decrease, and hydrocortisone (0.5 µg/ml) a 35% increase, in IGFBP-3. An IGF-II analog (leucine substitution at amino acid 27) with a markedly reduced affinity for the type 1 IGF receptor was 10- to 100-fold less potent than IGF-II in reducing IGFBP-3. No proteolyzed fragments of IGFBP-3 were identified. These comparative potencies of IGF, analog, and insulin on IGFBP-3 levels are consistent with inhibition via the type 1 IGF receptor, while EGF reduces IGFBP-3 by a different mechanism. In comparison, levels of IGFBP-4 were slightly increased by these growth factors, and the 33- and 28-kD IGFBPs were increased by IGF and insulin, but not EGF. We speculate that keratinocyte responsiveness to IGF, insulin, and EGF may be mediated in part by effects on IGFBP levels.

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LOW DOSE ULTRAVIOLET LIGHT INDUCES N-ACETYLTRANSFERASE ACTIVATION IN CULTURED HUMAN EPIDERMAL KERATINOCYTES. Yo Kawakubo, Mariko Iizuka, Itsuro Matsuo, Muneo Ohkido, Department of Dermatology, Tokai University School of Medicine, Isehara, Japan

Cutaneous exposure to UVB radiation is associated with biological effects including carcinogenesis. There were some reports on the effect of UVB on the epidermal metabolism, however, whether the acetylation, known as an activating reaction of procarcinogen, is affected by UVB irradiation remained to be investigated. Using cultured human epidermal keratinocytes (HEK), we have investigated effects of low dose UVB irradiation on the epidermal N-acetylating activity. HEK were irradiated by a FL-20SE lamp. At 24 hours after irradiation, HEK were harvested and homogenized, then centrifuged (9000 X g, 30 min) to get S9 fraction which is subject for assay. N-acetylated aminofluorene was detected by HPLC. UVB irradiation (200 J/m²) elevated significantly ($p < 0.01$) the activity from 2.33± 0.34 to 4.13 ± 0.89 nmol/mg protein/min, respectively. On kinetical studies, increase in Vmax (irradiated: 6.1, control: 2.2 nmol/mg protein/min, respectively) but little difference in Km value (irradiated: 81, control: 55 µM, respectively) were observed. Dose-response relationship was also studied from 80 to 360 J/m² with a maximized effect at 240 J/m². From a time course experiment up to 72 h after irradiation, the highest activity was observed at 48 h. In summary we have elucidated the effect of UVB irradiation on the epidermal acetylating capacity for the first time, suggesting that the low dose UVB irradiation may affect the sensitivity of the epidermis towards arylamines including procarcinogens.

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GROWTH AND DIFFERENTIATION PROPERTIES OF NORMAL HUMAN KERATINOCYTES IN ORGANOTYPIC CULTURE. M. Tsunenaga, I. Horii, Y. Kohno*, E. Adachi** and T. Kuroki*, Skin Biological Research Laboratories, Shiseido Research Center, Yokohama, *Department of Cancer Cell Research, Institute of Medical Science, University of Tokyo, Tokyo, and **Department of Anatomy, Osaka University Medical School, Osaka, Japan

Organotypic culture of skin is unique in that keratinocytes form a 3-dimensional structure on the top of contracted collagen gel containing fibroblasts. We investigated growth and differentiation properties of keratinocytes grown in organotypic culture.

Keratinocytes formed multilayers consisting of basal cell layer, spinous cell layer, granular cell layer and cornified layer. BrdU-incorporated cells were localized in the basal cell layer. Cells in differentiated cell layers expressed involucrin, filaggrin and transglutaminase. Basal cells expressed constituents of basement membrane, i.e. type IV-, type VII-collagen and kalinin on the border of the keratinocyte sheet and collagen gel, but electron microscopically only an incomplete structure of basement membrane was formed. When fibroblasts in the collagen gel were killed, growth of keratinocytes was much suppressed, but restored by the use of conditioned medium. HGF (hepatocyte growth factor) could not replace for the conditioned medium.

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VASOACTIVE INTESTINAL PEPTIDE (VIP) SUPPORTS MIGRATION AND GROWTH OF HUMAN KERATINOCYTES ON POLYURETHANE FOILS. Uwe Wollina, Brunhilde Knöll and Karin Prüfer, Department of Dermatology, Friedrich Schiller University Jena, Germany

Vasoactive intestinal peptide (VIP) has been shown to be a growth factor for the human keratinocyte cell line HaCaT in vitro [Neuroendocrinol Lett 14: 21-31, 1992]. We explored the possible role of VIP in HaCaT migration using an in vitro wound healing model. Briefly, HaCaT cells were seeded on chamber slides partially covered by an adhesive polyurethan dressing (PD). Cells were cultured under serum free conditions and exposed to various concentrations of VIP (1 fM to 1 μM) 48 h to 144 h. Medium and VIP were changed every second day. The PD itself had no significant effects on cell number, IL6 and TPS secretion by HaCaT's. We assessed both the migration of cells on the PD and their outgrowth from the free wound edge on the "wounded" slide surface. VIP free controls disclosed almost no migration onto the PD, though the keratinocytes neighbouring the dressing had growth characteristics as usual. On the other hand, VIP treated samples disclosed subconfluency to confluency on PD's after 48 h in a dose dependent manner. VIP had profound effects on cell morphology, too. Growth after wounding was significantly supported by 1 nM VIP showing a maximum in 96 h cultures (212 % of controls, $p < 0.01$). Cells migrated from the free wound edge in a chain-like pattern in contrast to samples treated with 1 μM VIP or controls showing protruding sheets. In conclusion, VIP has profound effects on HaCaT migration in vitro and facilitates growth and survival of keratinocytes on polyurethane dressings in vitro.

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A COMPARATIVE STUDY OF CYTOTOXICITY OF SKIN IRRITANTS BETWEEN CULTURED HUMAN ORAL AND SKIN KERATINOCYTES. Jin Ho Chung, Hee Chul Eun, Seung Yong Jung and Kwang Hyun Cho, Department of Dermatology, College of Medicine, Seoul National University, Seoul, Korea

The cytotoxicity of irritants using human oral and skin keratinocytes culture models was compared. Keratinocytes were exposed to sodium lauryl sulfate and benzalkonium chloride at concentration of 10^{-4} M - 10^{-7} M for 24 h. Cytotoxicity was evaluated by changes in mitochondrial metabolic activity (MTT assay) and plasma membrane integrity (LDH leakage). Our results showed that oral and skin keratinocytes are equally sensitive to the irritants tested. There were marked similarities in susceptibility between each cell line cultured from six individuals. The immunohistochemical staining pattern of both cell types resembled that of the basal cell.

These observations suggest that the skin keratinocytes culture model may be useful in evaluating the cytotoxicity of irritants related to skin and oral mucosa.

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PHORBOL ESTER CAUSES A RAPID WITHDRAWAL OF PRIMARY HUMAN KERATINOCYTES FROM THE CELL CYCLE. M. R. Green, *A. J. Wallace, *R. F. Brooks, Biosciences Division, Unilever Research, Sharnbrook, Bedford, UK; *Anatomy and Human Biology Group, Kings College, London, UK

The phorbol ester 12-0-tetradecanoylphorbol-13-acetate (TPA) is a pharmacological activator of the PKC family of proteins and, when added to human keratinocytes, causes decreased cell clonogenicity, proliferation and accelerated differentiation. This study examined the detailed dose response and kinetics of withdrawal from the cell cycle of keratinocytes and transformed epithelial cell lines measured by BrdU labelling. Keratinocyte withdrawal commenced rapidly (<2h) and was complete within 11h of TPA application. In contrast, the synthetic diacylglycerol, OAG, had no effect on keratinocyte cell cycle withdrawal. A small population of keratinocytes (<1%) was resistant to TPA, and possessed properties consistent with a 'stem' cell phenotype. None of the three transformed cell lines tested (SCC 146, SVK14, HeLa) showed cell cycle withdrawal response to TPA. It is concluded that epithelial cell responses to PKC activators are complex but distinct and depend on cell phenotype and PKC activator.

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KERATINOCYTE DIFFERENTIATION IS INDUCED BY CELL-PERMEANT CERAMIDES BUT INHIBITED BY SPHINGOSINE. Hisashi Wakita, Yoshiki Tokura, Hiroaki Yagi, Fukumi Furukawa and Masahiro Takigawa, Department of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, Japan

Ceramide and sphingosine have been suggested as intracellular modulators of cell growth and differentiation. The effects of these sphingolipids on the growth and differentiation of keratinocytes were examined by using cultured human keratinocytes(a cell line squamous cell carcinoma, DJM-1 cell). Synthetic short-chain, cell-permeant analogs of ceramides, N-acetylsphingosine, N-hexanoylsphingosine and N-octanoylsphingosine significantly promoted the differentiation of cultured cells confirmed by the upregulation of the formation of cornified envelope, the synthesis of involucrin and the transglutaminase activity, and inhibited the proliferation exemplified by the reduction of cell numbers, DNA amount and ^{3}H-thymidine incorporation. Generally, these activities were augmented with extending N-acyl carbon chains. On the other hand, sphingosine(dihydroxysphingenine) at an appropriate concentration modestly stimulated the proliferation but inhibited the differentiation of cultured cells. Our results suggest the possibility that the growth and differentiation of keratinocytes are at least partially regulated by the counteraction of ceramide and sphingosine.

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OSTEONECTIN EXPRESSION DURING BUTYRATE-INDUCED DIFFERENTIATION OF CULTURED HUMAN KERATINOCYTES: REGULATION VIA TGF-β. G. Wang, R. Ford, P. Higgins and L. Staiano-Coico, Depart. of Surgery, Cornell University Medical College, New York, NY (USA) and Depart. of Microbiology, Albany Medical College, Albany, NY (USA).

Expression of osteonectin, a 43 kDa extracellular matrix (ECM)-associated glycoprotein involved in tissue remodeling, was quantitated during normal human keratinocyte (NHK) growth in culture and as a function of sodium-N-butyrate (NaB)-induced differentiation to mature enucleate cornified envelopes (CEs). Low levels of osteonectin expression were observed in the basal-like cells of control NHKs.After addition of NaB, osteonectin expression increased and the pattern of expression shifted to predominantly suprabasal cells. Dense deposits of osteonectin often surrounded the mature CEs. Flow cytometric (FCM) analysis indicated that ~13% of NHKs expressed osteonectin within 24 hr of seeding into culture.This fraction of osteonectin$^+$ cells increased with time and peaked immediately post-confluence (31.3±6.3%) and then decreased to baseline levels during plateau phase growth.Osteonectin levels were more intense and heterogeneous within the G_2/M and G_1 phases while S phase cells exhibited relatively homogeneous, low intensity,osteonectin expression. During NaB-induced NHK differentiation, osteonectin intracellular content increased prior to the onset of CE formation followed by extracellular accumulation which coincided with maximal CE generation. Correlation of cell size with osteonectin expression revealed a predominance of osteonectin in larger cells. Induced osteonectin expression was dependent on autocrine TGF-β production since incubation in the presence of NaB + neutralizing antibodies to TGF-β inhibited the intracellular expression of osteonectin by ~93%.

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EXPRESSION OF HETEROGENOUS CYTOPLASMIC GRANULES IN ABNORMALLY KERATINIZED EPITHELIUM. Motomu Manabe, Hitoshi Yaguchi, Michael W. O'Guin*, Tung-Tien Sun*, Hideoki Ogawa, Department of Dermatology, Juntendo University School of Medicine, Tokyo, Japan, *New York University School of Medicine, New York, U.S.A.

Filaggrin and trichohyalin are the two major keratin filament associated proteins in the skin. These two proteins initially accumulate in keratohyalin or trichohyalin granules which provide prominent morphological hallmarks of differentiation in the epidermis and the inner root sheath of hair follicles, respectively. Recently, we have reported that, in dorsal tongue epithelia, filaggrin and trichohyalin co-existed within a single, heterogenous granules. Subsequent to identifying the novel cytoplasmic granules, keratohyalin-trichohyalin hybrid granules, we have been able to demonstrate the existence of the hybrid granules in a number of hyperplastic epidermis. Further, our latest data revealed an additional level of complexity in the heterogenous cytoplasmic granules. In a subpopulation of keratinocytes suffered from molluscum contagiosum, interestingly, we found abundant expression of newly discovered cornified envelope precursor, loricrin, as well as filaggrin and trichohyalin. Understanding the structural and funcional interaction occurring between these three proteins should provide valuable insght into the very late stage of both normal and abnormal epithelial differentiation.

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DOWN-REGULATION OF INTERFERON-GAMMA INDUCED ICAM-1 AND HLA-DR EXPRESSION ON KERATINOCYTES BY ANTIPSORIATIC DIMETHYL-FUMARATE.

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The oral administration of complex mixtures of fumaric acid derivatives is effective in the treatment of psoriasis. Most of this antipsoriatic potential seems to derive from the antiproliferative dimethyl-fumarate (DMF) compound (J Invest Dermatol 100: 467, 1993). Concerning the dysregulation of epidermal cell surface markers in psoriasis the present study focused on the effect of DMF on the interferon-gamma (IFN-γ) induced expression of ICAM-1 and HLA-DR on keratinocytes.

Hyperproliferative HaCaT keratinocytes were simultaneously exposed to IFN-γ (10 U/ml) and DMF (1.3 μM, 4.00 μM) for 48 hours. For the detection of ICAM-1 and HLA-DR expression an ELISA-APAAP technique was set up and performed directly in 96-well plates. The absorbance of the final p-nitrophenol reaction product was related to the cell number per well a total of 24 measurements for each incubation in 4 independent experiments).

The results were as follows (mean±SD; asterisks indicate significant effects, $p \leq 0.05$, as compared to the controls treated with IFN-γ alone):

	Cell number	ICAM-1	HLA-DR
Control	100.0±4.3	100.0±13.0	100.0±8.9
DMF 1.3 μM	96.9±4.1*	97.9±16.7	82.9±10.7*
DMF 4.0 μM	94.4±4.0*	83.7±17.7*	66.7±10.7*

Thus the IFN-γ induced expression of ICAM-1 and HLA-DR on hyperproliferative keratinocytes was significantly suppressed at subtoxic DMF concentrations of 4.0 and ≥1.3 μM, respectively. Therefore it may be concluded that DMF exerts its antipsoriatic efficacy not only by its known antiproliferative activity but also by down-regulation of cell adhesion molecules.

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KERATIN MUTATIONS IN EPIDERMOLYTIC HYPERKERATOSIS PROVIDE A MODEL FOR KERATIN INTERMEDIATE FILAMENT INSTABILITY.

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Epidermolytic hyperkeratosis (EH) is an autosomal dominant disorder affecting the structural integrity of the keratin intermediate filaments (KIF) of the suprabasal layers of the epidermis. Gene linkage studies and mutational analyses have now established that mutations in the genes of the terminal differentiation specific keratins K1 (type II) and K10 (type I), which result in inappropriate amino acid substitutions, cause this disease. Nor is it yet clear whether there is a relationship between disease and the type of mutation and its structural implications for KIF. To address this question, we have identified 10 additional mutations in separate probands of EH. Six mutations have been observed at the start of the rod domain segment 1A of the K10 chain: N8 to H; R10 to C, R10 to H (three cases); and Y14 to D. One other mutation in K10 occurs in the 2B rod domain segment, L103 to Q. Three other mutations have been identified in the K1 chain: V11 to G of the H1 end domain segment; N8 to S and S13 to P of the 1A rod domain segment. Each of these mutations has been tested in an in vitro KIF disassembly assay. Synthetic peptides bearing the substitutions are less effective in disassembling preformed KIF than the wildtype peptide, indicating the important role of these residues in KIF structure. Together with our earlier data and that from other laboratories, we know of 19 mutations that cause EH. Likewise, a total of 9 mutations are known to occur in the K5 and K14 chains which cause epidermolysis bullosa simplex (EBS). Of these 28 mutations, 60% occur between residues 6 and 15 of the 1A rod domain segment, with all others scattered along the protein chains. Structural analyses reveal that 75% of the mutations occur in sequences involved in the head-to-tail overlap of molecules in KIF. Accordingly, our date provide: (i) a catalog which will aid in the identification of further mutations for EH or EBS, and be of use in genetic counseling; and (ii) a basis for solving the three-dimensional structure of KIF.

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CATHEPSIN L-SPECIFIC HIGH MOLECULAR WEIGHT CYSTEINE PROTEINASE INHIBITOR (PSORIASTATIN) FROM PSORIATIC SCALES.

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Changes of proteinases and their inhibitors appear to be correlated with disease activities. In the present study, we purified and characterized a 43 kDa cysteine proteinase inhibitor (psoriastatin) which was specifically found in psoriatic epidermis. A screening test for the psoriatic scale extract fractionated by Sephacryl S-200 gel filtration demonstrated an unidentified peak for cathepsin L inhibition in the high molecular weight range. This was distinguished from cystatin in terms of molecular weight and reaction with anti-cystatin IgG. Purified psoriastatin migrates as a single 43 kDa protein. It specifically inhibited cathepsin L with a Ki of 1.9 nM. It had no inhibitory effect on cathepsin B or H. Complex formation between psoriastatin and cathepsin L was investigated at different pH values. The 43 kDa band, native form, partially shifted to 35 kDa when the complex was formed at pH 5.0. It means that the equilibrium favors the complex in which the inhibitor has its peptide bond cleaved. At pH 6.0 or above, the complex remains in its initial Michaelis-type state, suggesting that psoriastatin act as cathepsin L inhibitor in physiological condition. Immunohistochemical studies revealed that the psoriastatin first appeared in the nuclei of cells above the basal layer and then diffused into the lysosome in accordance to the progression of the psoriatic condition. Partial amino acid sequence showed a close homology to SCC antigen which belongs to the serpin family. However, psoriastatin does not inhibit any serine proteinases. Since the SCC antigen is found in various inflammatory tissues, psoriastatin may be involved in the inflammatory response in psoriasis.

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HUMAN EPIDERMAL SPHINGOMYELINASE IS DECREASED IN THE SKIN OF THE AGED AND ATOPIC DERMATITIS PATIENTS.

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Sphingomyelinase(SPase) is the enzyme which hydrolases sphingomyelin into ceramides and phosphorylcholine, and its enzymatic activity was decreased with aging [Yamamura T and Tezuka T, 1990]. The purpose of this study is to determine the amount of SPase in the epidermis immunohistochemically and to compare its amounts between the aged and the young, or the normal and the atopic dermatitis patients semiquantitatively.

A polyclonal antibody to human epidermal SPase has been prepared by two steps; Firstly, monoclonal antibodies for commercially available human placental SPase were prepared. Among them, the clone, the cultured supernatant fraction of which inhibited not only SPase activity, but also cross-reacted with the stratum granulosum cells of human epidermis, was selected. This monoclonal antibody recognized a 60 kD epidermal protein. Secondly, a polyclonal antibody(SK-1) for this 60kD epidermal protein has been prepared. SK-1 inhibited SPase activity of the epidermal homogenate. The abundant reaction product with SK-1 was observed in the stratum ganulosum and the innermost cell layers of the stratum corneum of the normal control. In contrast, there was only a trace of products occurred in the epidermis of the lower leg skins of the aged and a small amount of products in atopic dermatitis patients skins. The density of reaction products of the normal skin was obviously greater than those of the aged and atopic dermatitis skins, which was statistically significant.

The decrease of total amounts of ceramides in the stratum corneum of the atopic dermatitis patients, which was reported by Imokawa et al., and xerosis in the lower leg skin of the aged may be partly due to the possible decrease of ceramides synthesis being based upon the decrease in the amount of SPase.

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INFLUENCE OF FREE RESIDUAL CHLORINE ON CULTURED HUMAN KERATINOCYTES FROM NORMAL SKINS AND HYPERTROPHIC SCARS.

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In Japan, the water of a swimming pool in school is restricted to contain over 0.4 mg/l but desirably under 1.0 mg/l of free residual chlorine. In this study, the influence of free residual chlorine on cultured human keratinocytes of normal skin and hypertrophic scars was examined. MTT assay and flow cytometric technique were used to assess the toxicity to and the DNA content of keratinocytes. Free residual chlorine of 100 mg/l showed about 50% toxicity to the keratinocytes of normal skin, and even greater toxicity to those of hypertrophic scars. These findings suggest that free residual chlorine in the water of a swimming pool at a concentration of 100 mg/l may cause some damages to the skin and hypertrophic scars.

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EPIDERMIS RECONSTRUCTED FROM THE OUTER ROOT SHEATH OF HUMAN HAIR FOLLICLE ON DE-EPIDERMIZED DERMIS. EFFECT OF RETINOIC ACID.

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Reconstruction of human epidermis on a dermal equivalent made of collagen type I and living fibroblasts has been interpreted as a phenotypical transition of outer root sheath cells. It was interesting to assess the possible role of living fibroblasts in this process. This was achieved by cultivating human hair follicles, on de-epidermized dermis (Pruniéras et al. 1979, Ann. Chir. Plas. 24, 357-362).

A multilayered and well differentiated epidermis can be obtained by 15 days. Histologically, this epidermis presented characteristic features of normal human epidermis in vivo. A complete basal membrane with numerous hemidesmosomes was seen. The basal cells were well oriented with their main axis perpendicular to the dermo-epidermal junction. Spinous and granular layers could be observed, with numerous keratohyalin granules. Cornified layers laid on top of the cultures. Moreover, markers specific for interfollicular keratinocyte terminal differentiation, such as the K 10 keratin, involucrin, membrane-bound transglutaminase, filaggrin and loricrin, were expressed in the reconstructed epidermis. By in situ hybridization, keratin K 5 and K 10 mRNAs were localized in the basal and suprabasal cells respectively, like in normal human epidermis. These results demonstrate that the differentiation pathway of human outer root sheath cells is intrinsically labile and can be shifted towards the interfollicular keratinocyte pattern by air exposure, even in the absence of living dermal fibroblasts. The differentiation of the reconstructed epidermis is modified by retinoic acid treatment, in a dose-dependent manner. This culture system on dead dermis is easier to handle than similar cultures on collagen-fibroblast lattice because of the resistance of dermis to mechanical forces and to collagenolysis. It could represent a valuable wound-healing model and a promising tool for pharmacological studies on in vitro reconstructed skin.

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MURINE "HAIR-RELATED" KERATINS MHRa1 AND MHRb1 ARE USEFUL MARKERS FOR THE EVALUATION OF THE BIOLOGICAL ACTIVITY OF ORALLY ADMINISTRATED SYNTHETIC RETINOIDS

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Differentiating cells of the parakeratinizing regions of adult mouse tail epidermis express the "hair-related" keratin pair MHRa1 (48 KD) and MHRb1 (65 KD) whose synthesis is completely suppressed by daily topical treatment of tail epidermis with all-trans retinoic acid (RA) in a time and dose-dependent manner concomitant with an orthokeratotic conversion of the epidermis (Tobiasch et al. Differentiation 50, 163-171, 1992). In the present study we have investigated whether both orthokeratotic conversion and suppression of MHRb1 mRNA synthesis is also brought about after oral administration of retinoids currently used in dermatology by this route (Roaccutane for acne and Soriatane for psoriasis). Groups of 4 mice received oral daily doses of 30, 100 and 300 mg/kg body weight of RA, Roaccutane and Soriatane for two weeks. RNA was isolated from 1 cm^2 tail epidermis, spotted in 5 and 2 µg aliquots to nylon membranes and hybridised with a digoxigenin labelled specific cDNA probe of MHRb1. After staining with NBT and X-phosphate the dots were scanned and the percentage inhibition of MHRb1 mRNA synthesis was expressed relative to untreated control. All three RA concentrations led to a suppression of about 60%. In contrast, both Roaccutane and Soriatane suppressed the MHRb1 mRNA synthesis in a dose dependent manner with a maximal inhibition of 42% and 53% respectively. No histological changes were observed in tail epidermis of animal treated with 30 mg/kg of the three retinoids. The orthokeratotic conversion of the tail epidermis was clearly observed in the 100 and 300 mg/kg groups treated with all three retinoids. These results show that mouse "hair-related" keratins are useful and highly sensitive *in vivo* markers for a simple and rapid evaluation of the biological activity of topically and/or orally administered synthetic retinoids. Oral retinoids may have similar molecular targets despite the fact that they are used to treat different diseases.

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CHARACTERIZATION OF PHOSPHORYLATED CYSTATIN α IN NEWBORN RAT EPIDERMIS.

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We have isolated a cysteine proteinase inhibitor, which is hematoxylin stainable and is located in keratohyalin granules and cornified envelope, from newborn rat epidermis. The properties of this cysteine proteinase inhibitor were similar to those of a recombinant cystatin α in molecular weights, amino acid compositions, and inhibitory activities against several proteinases; however, isoelectric points of these proteins were different: the former's pI was 4.7 and the latter's pI was 5.3. In addition, the recombinant cystatin α was phosphorylated by protein kinase C. For further characterization of phosphorylated cystatin α (P-cystatin α), the followed experiments were performed. P-Cystatin α was reacted with alkaline phosphatase and the reacted materials were analyzed by 2-dimensional electrophoresis and immunoblotting technique using anti-P-cystatin α Ab. P-Cystatin α was incubated in lysyl endopeptidase, the reacted material was subjected to a reverse phase high-performance liquid chromatography (HPLC), and then, each fraction was collected. The absorbance of each fraction based on the complex of phosphated, molibdate and Malachite Green was measured at 660 nm. The protein spot produced by alkaline phosphatase reacted with anti-P-cystatin α Ab, and its isoelectric point coincided with that of the recombinant cystatin α. The HPLC fraction containing T-K and T-N-E-K peptides showed the highest absorbance. Therefore, these findings suggest that the protein which is dephosphoryl by alkaline phosphatase is a derivative of P-cystatin α and the phosphorylated amino acid in P-cystatin α could be threonine of the T-K or T-N-E-K peptide.

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EICOSAPENTAENOIC ACID INDUCED CHANGES IN KERATINOCYTE MEMBRANE FLUIDITY IN VITRO.

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The effect of eicosapentaenoic acid(EPA, 20:5n-3) on the membrane lipid fluidity of cultured human epidermal keratinocytes was investigated using the method of fluorescence recovery after photobleaching(FRAP). When the keratinocytes were treated with 3 µg/ml of EPA for 72 h, a significant amount of EPA was found to be incorporated into the cells. After the treatment with EPA, the membrane lipid fluidity was evaluated by measuring the diffusion coefficient using the method of FRAP, which was performed at an ACAS 470 work station. Lipophilic fluorophore diphenylheatrien(DPH) was used as a probe. For the FRAP analysis, only small undifferentiated keratinocytes were used. The diffusion coefficient of the untreated keratinocytes was revealed to be $1.41 \pm 0.38 \times 10^{-8} cm^2/sec$(n=71). Treatment of the cells with EPA resulted in the increase of the coefficient to $1.90 \pm 0.49 \times 10^{-8} cm^2/sec$(n=55)($p<0.005$). There was no obvious difference in the recovery rate between these experiments: untreated cells; 32.0 ± 9.3 %, and EPA treated cells; 31.5 ± 9.1 %. These results indicate that EPA could be incorporated into the cells and subsequently increase their plasma membrane fluidity. This process might be associated with the modulatory effect of EPA in the growth of human epidermal keratinocytes and may relate to its clinical effect on psoriasis.

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THE ROLE OF PROTEASES IN STRATUM CORNEUM: INVOLVEMENT IN STRATUM CORNEUM DESQUAMATION.

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Corneocytes are shed from the skin surface successively. However, the mechanism of stratum corneum (SC) desquamation has not thoroughly understood. We previously detected two types of serine-proteases in SC, namely, trypsin-type (30 kDa) and chymotrypsin-type (25 kDa) proteases, and this study was designed to clarify this involvement in SC desquamation. We examined the cell dissociation process from SC sheet in detergent solution and the desmosomal digestion in SC sheet. In detergent solution SC sheet was dissociated into individual cells. Serine-protease inhibitors retarded the cell dissociation; leupeptin and chymostatin each reduced the cell dissociation about half as effectively as aprotinin, while a mixture of the two prevented SC sheet degradation as potently as did aprotinin or antipain. Immunochemical measurement of desmosomal protein indicated that desmosomes were present in intact SC sheet, but they were not detected in the dissociated cells. These results suggest that both trypsin- and chymotrypsin-type serine-proteases act on desmosome and they are involved in SC desquamation.

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IN SITU HYBRIDIZATION OF PROELAFIN, A NEW INHIBITOR OF LEUKOCYTE ELASTASE

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The activity of leukocyte elastase (LE) is regulated by circulating and locally produced elastase inhibitors. Defficiency of α-1-antitrypsin, an important circulating inhibitor, leads psoriasis, value of inhibitors is emphasized. From psoriatic scales, elafin, a new inhibitor of LE, was purified by Wiedow et al. But there has been no evidence that elafin is produced in mormal epidermis, the physiological and pathological role of elafin remains unknown. We determined proelafin mRNA expression in normal human epidermis by in situ hybridization using a RNA probe for the second exon encoding the sequences for 'pro' and mature elafin. Psoriatic epidermis, the surface of which shows high LE activity, was also examined. Proelafin mRNA was expressed in the granular layer in normal epidermis. Psoriatic epidermis showed abundant and extensive expression of proelafin mRNA compared with normal epidermis, elafin is suggested to play an important role against high level of LE in psoriasis.

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A SOLUBLE FACTOR PRODUCED BY DERMAL INFILTRATE OF CUTANEOUS T CELL LYMPHOMAS INHIBITS THE EXPRESSION OF AN EPIDERMAL ANTIGEN : MY 7.

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My7 antigen (CD13) is expressed in basal cells of normal epidermis and previously we have demonstrated that this expression specifically disappeared, in cutaneous lesions of epidermotropic cutaneous T cell lymphoma (CTCL) both in vivo and in vitro and could be induced by Interferon α (IFNα) again. In this work, we tried to determine the role of tumoral lymphocytes in the abolition of this My7 expression.

So we have extracted lymphocytes from 5 CTCL lesional skins and 2 inflammatory skin lesions (eczema) used as control (histological control performed before). Then after in vitro proliferation, theses lymphocytes were incubated for one week with reconstituted skins (RS) obtained from normal skin previously controled for positive My7 antigen expression. In a similar manner, RS were incubated with the surnageant of lymphocytes culture medium.

Our study shows that tumoral lymphocytes (CD4 phenotype) of all 5 patients inhibit My7 expression in basal cells of RS. Moreover, the 5 surnageants of culture also inhibit My7 expression. On the contrary, no abolition of My7 expression is noted with CD4 reactional lymphocytes of the 2 eczematous lesions. Interestingly, the addition of IFNα [100UI/ml] in the medium of RS incubated with CD4+ tumoral lymphocytes or surnageant partially induces My7 expression in basal cells again.

In conclusion, abolition of My7 expression in basal cells is related to a soluble factor produced by the lymphocyte infiltrate of CTCL whose effect can be partially inhibited by IFNα. Moreover, this study demonstrates an interaction between keratinocyte and T lymphocyte in epidermotropic CTCL.

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EPIDERMAL KERATINOCYTES EXPRESS CARCINOEMBRYONIC ANTIGEN (CEA) GLYCOPROTEINS. Metze Dieter[1], Bhardwaj Ranschit[2], Amann Udo[1], Artuç Metin[3], Kolde Gerd[1], Luger Thomas[2], Grunert Fritz[4]. [1]Dept. Dermatol., [2]LBI-DVS,Lab Cell Biol., Univ. Münster, [3]Dept. Dermatol., Univ.R.-Virchow,Berlin, [4]Inst. for Immunobiol.,Univ. Freiburg, FRG.

CEA has been demonstrated in skin glands and cutaneous tumors. To examine the expression of CEA glycoproteins in epidermal keratinocytes a panel of highly specific anti- CEA antibodies was applied for Western blot analysis or RIA,light and electronmicroscopic immunostaining or confocal laser scanning microscopy (CLSM). In the human epithelial cell lines A 431 and HaCaT various glycoproteins of the CEA family were constitutively expressed and could be upregulated by interferon gamma. Immunoelectronmicroscopy revealed CEA in the Golgi apparatus, intracytoplasmic vesicles and along the cell surface. CLSM of cultured monolayers revealed a staining of CEA confined to the intercellular plasma membranes. CEA was neither detectable in the KB line, human normal keratinocytes, a melanoma line nor in normal human epidermis. In fetal skin, a skin equivalent model or during wound healing keratinocytes expressed CEA at their cell surface in the upper epidermal cell layers. A similar staining pattern was seen in pseudoepitheliomatous hyperplasia and viral warts. Corresponding but more inconsistent results were obtained in a variety of inflammatory diseases. Generally, CLSM and electronmicroscopy proved CEA glycoproteins confined to the cell surface of the keratinocytes. In conclusion,the expression of CEA is closely related to differentiation or cellular polarity and indicates a function of CEA for cell surface recognition of keratinocytes during embryonic development or hyperproliferative and inflammatory conditions.

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DISEASE ASSOCIATED KERATIN MUTATIONS: REPRODUCTION OF PHENOTYPE *IN VITRO*. I M Leigh[1], C J Sexton[1], H A Navsaria[1], R A J Eady[2], [1]Experimental Dermatology, London Hospital Medical College, London, UK; [2]St John's Inst. Dermatology, UMDS, St Thomas' Hospital, London, UK.

Point mutations in keratin genes in conserved regions critical for filament assembly can produce abnormal cytoskeletal networks in a dominant disruptor fashion, when introduced into keratinocytes in vitro or in transgenic mice. Molecular genetics with linkage analysis, biochemical evidence and cDNA sequencing have now converged to show conclusively that mutations in keratins K5 and K14 are found in all forms of epidermolysis bullosa simplex (EBS) and mutations in keratins K1 and K10 are found in all forms of epidermolytic hyperkeratosis (EH). Cultured keratinocytes have been established from multiple families with EBS, EH and tylosis, immortalised by treatment with SV40 T or HPV 16 and then cultured past crisis to establish keratinocyte lines bearing individual known keratin mutations. Keratinocytes are established in organotypical culture using collagen gels or de-epidermalised dermis as substrate when stratifying cultures with improved differentiation are obtained. Primary keratinocytes show subtle and infrequent cytoskeletal abnormalities, when cultured on plastic substrate, but differentiating organotypical cultures show increased cytolysis in EBS-Dowling Meara. Low levels of K1 and K10 occur even in stratifying cultures of EH. Immortalised lines show induction of simple epithelial keratins, more readily in SV40 lines, which appear to form a stable cytoskeleton. Primary tylosis keratinocytes show major disruption of keratinocyte cytoskeleton with perinuclear clumps and loss of the perinuclear filament condensation. Thus subtle disease associated phenotypic characteristics can be reproduced *in vitro* in EBS and tylosis.

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CHANGES OF CYTOSKELETONS, EXTRACELLULAR MATRICES AND EXTRACELLULAR MATRIX RECEPTORS IN CUTANEOUS SQUAMOUS CELL CARCINOMA. Miyoko Kubo, Shinichi Ohno*, and Toshiaki Saida, Department of Dermatology, Shinshu University School of Medicine, Matsumoto; *Department of Anatomy, Yamanashi Medical University, Yamanashi, Japan

The mechanism by which squamous cell carcinoma (SCC) grows and/or metastasizes is mostly unknown. To analyze the factors that influence the invasion and metastasis of cutaneous SCC, we investigated the changes of cytoskeletons, extracellular matrices (ECMs) and extracellular matrix (ECM) receptors in 3 cases of SCC (2 well differentiated SCC and 1 poorly differentiated SCC) and compared the findings with those of normal skin.

Significantly increased amount of F-actin were demonstrated in all cases of SCC by FITC-phalloidin staining. Investigation with indirect immunofluorescence technique showed that the number of high molecular weight cytokeratin (68 kD)-positive cells were decreased in all cases of SCC and vimentin-positive cells were detected in a poorly differentiated SCC. The expression of laminin and laminin receptor were increased in all cases of SCC, accompanied with increased amount of fibronectin in the surrounding stroma. Ordinary electron microscopic observation revealed increased amount of microfilaments, increased number of cytoplasmic processes and microvilli, and decreased number of desmosomes. Focal destruction of basal lamina was also detected. Three-dimensional observation by quick-freezing and deep-etching method showed increased amount of microfilaments.

These data suggested that the increase of microfilaments and the decrease of desmosomes in SCC may contribute to the invasion of tumor cells into surrounding tissues. The changes of ECMs and ECM receptor revealed in the present study may have important meanings in the biologic behaviors of SCC.

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K1 KERATIN PEPTIDE IS A WIDESPREAD COMPONENT IN CULTURED HUMAN SQUAMOUS CELL CARCINOMAS. Yohtaro Katagata, Yutaka Hozumi and Shigeo Kondo, Department of Dermatology, Yamagata University School of Medicine, Yamagata, Japan

Keratins, epithelial intermediate filament proteins, consist of 20 proteins (K1-K20) and are divided into two families, acidic- and basic-types. K1 / K10 is a characteristic keratin pair of suprabasal keratinocytes and may be essential to construct and maintain the structural and functional requirements during terminal differentiation. To date, the largest keratin peptide (K1, 68 Kd) has been absent in cultured human squamous cell carcinomas (HSC). Using a low salt aqueous solution, not containing high salt / Triton X-100, as a washing buffer for keratin extraction, followed by two dimensional polyacrylamide gel electrophoresis, immunological techniques and Northern blot analysis, we demonstrated K1 peptide in six kinds of cultured HSCs. Until now, keratin extraction has been done using high salt / Triton X-100 solution, during which K1 peptide may be removed because it has developed an affinity with the buffer. Moreover, the K1 peptide was a little unusual with respect to solubility versus urea concentration. Namely, epidermal K1 peptide is usually solubilized by 6-8 M urea / reductant, however, the K1 peptide in cultured HSCs was about 80-90 % extracted in 1-2 M urea /reductant by a step wise extraction procedure. On the other hand, K10 peptide, which is the pair-keratin with K1, was not expressed in HSCs employed. So, the K1 peptide may exsist itself only, not form keratin filament in cultured HSCs.

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CENTROMERE PROTEIN B IS HIGHLY EXPRESSED IN PSORIATIC SKIN.

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The Centromere/kinetichore is a specialised structure located at the primary constriction of eukaryotic chromosomes and functions to attach chromosomes to mitotic spindle. It is required for normal movement and disjunction of chromosomes during mitosis and meiosis. Centromere protein B (CENP-B) constitute the most important part of centromere. Psoriasis is a common skin disease with unknown aetiology and it is characterised by hyper-proliferation of epidermis. In order to explain the pathologic cell kinetics in psoriasis, we investigated CENP-B mRNA expression in normal and psoriatic skins. Total RNA were extracted from hyperproliferative lesions of psoriatics and normal skin of healthy volunteers and analysed by using RNA blotting hybridised with a ^{32}P-labelled cDNA probe. The result demonstrated that CENP-B mRNA was highly expressed in psoriatic skin compared with normal control. Moreover, CENP-B expression was also analysed in different phases of cell cycle in synchronised HeLa cell and shown to be expressed in G1, S, G2 and M phase but, especially, elevated during G2 phase. Therefore, it is indicated that elevated CENP-B expression is an important event in the pathophysiology of psoriasis.

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Tyr-Ile-Gly-Ser-Arg (YIGSR) PEPTIDE IN LAMININ PROMOTES THE TERMINAL DIFFERENTIATION OF KERATINOCYTES. Keizo Yamamura, Motoyoshi Nomizu, Masamitsu Ichihashi, and Hynda K. Kleinman, Lab. of Developmental Biology, National Institute for Dental Research, NIH, Bethesda, MD, USA (KY, MN, HKK) and Dept of Dermatology, Kobe Univ. School of Med., Kobe, Japan (KY, MI)

Laminin, a major constituent of basement membrane, has various biological activities with several active sites defined at the synthetic peptide level. Whole laminin is reported to have no effects on keratinocyte terminal differentiation induced by suspension in methylcellulose, whereas fibronectin is reported to inhibit the differentiation.

In order to reveal the functions of respective active sequences in laminin for keratinocyte terminal differentiation, synthetic peptides containing active sites, Tyr-Ile-Gly-Ser-Arg (YIGSR), Ser-Ile-Lys-Val-Ala-Val (SIKVAV), Arg-Gly-Asp (RGD), and related peptides were prepared and assayed.

Neonatal human foreskin keratinocytes were cultured in keratinocyte growth medium with or without these synthetic peptides, and antibodies to involucrin and filaggrin were used as markers of differentiation.

Whole laminin showed no effect as already reported. Neither SIKVAV, RGD, nor other related peptides tested in this study had effect on keratinocyte differentiation. Only the YIGSR peptide showed the differentiation promoting effect at the concentration of 30 - 100 μg/ml. These data demonstrate that a specific site on the laminin may at certain stages regulate keratinocyte differentiation.

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IMMUNOHISTOCHEMICAL CHARACTERIZATION OF THE HUMAN NAIL MATRIX IN VIVO.

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The nail matrix is a specialized epithelial structure with peculiar anatomical and physiological properties. The aim of our study was to evaluate the cell-cell, cell-matrix and cell basement membrane interactions in the nail matrix as compared with their epidermal counterparts.

The nail biopsies were obtained from 2 autopsy fingers and from 8 patients with ingrown toenails. The basement membrane zone (BMZ) antigenic characteristic were studied using the following antibodies: anti-type IV collagen, anti-type VII collagen, anti-laminin, anti-tenascin, anti-epiligrin, KF1, AHS-7, 19-DEJ-1, HD-121, LH 39, GB3, Bullous Pemphigoid and Herpes Gestationis Factor. The integrin expression was studied using anti-α1, anti-α2, anti-α3, anti-α4, anti-α5, anti -α6, anti-αv, anti-β1 and anti-β4 monoclonal antibodies. Immunofluorescence assay were performed on cryostat cut tissue sections. Biopsies from normal human foreskin were evaluated as controls.

The BMZ of the nail matrix expressed the same antigens of the BMZ of the human foreskin. The pattern of integrin expression of the nail matrix was similar to that of the human foreskin epidermis. However, in the nail matrix, expression of α2, α3 and β1 was not only detected in the basal layer (as in control skin), but also in 4 to 5 suprabasal cell layers (as in hyperproliferative epidermis), with a suprabasal expression decreasing gradually from the distal to the apical portion of the nail matrix epithelium.

Our findings show that despite the distinctive features of the nail apparatus compared to the epidermis, BMZ antigenic characteristics and pattern of integrin expression is similar, although some differences in the distribution of α2 , α3 and β1 subunits are detectable. These are probably related to the peculiar differentiation and keratinization of the nail.

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ROLE OF β1 INTEGRINS IN MELANOCYTE CELL SPREADING AND MIGRATION ON COLLAGEN TYPE IV AND LAMININ.

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We characterized β1 integrin receptor expression on three different cultures of human melanocytes by flow cytometry and examined their role in mediating cell spreading and migration on collagen type IV (CN IV) and laminin (LN) coated substrates by using a quantitative video image analysis system. The three human melanocyte cultures expressed β1 integrin (Mean Fluorescence Intensity; MFI = 316, 261 and 289 respectively), VLA-2 (MFI = 32, 90 and 68), VLA-3 (MFI = 29, 45, 72) and VLA-6 (MFI = 12, 21, 28). A significant increase ($p<0.001$) in cell spreading and migration of the melanocytes was induced on increasing coating concentrations of CN IV or LN. This extracellular matrix (ECM) protein-induced cell spreading and migration was significantly inhibited by anti-β1 mAb (AIIB2), anti-α2 mAb (P1E6), or anti-α3 mAb (P1B5), but not by mAb against α6 integrin subunit (GoH3). Image analysis determined that cell area significantly decreased with the anti-β1 mAb during migration on CN IV but not on LN. In contrast, the anti-α2 mAb induced a decrease in melanocyte cell area on LN but not on CN IV. The other mAbs had no significant effect on cell area. However, cell form factor increased following treatment of each mAb, indicating partial retraction of cell processes, but not detachment of the cells. These data suggest that α2β1 and α3β1 cell adhesion molecules are important for melanocyte cell spreading, maintenance of cell processes, and migration on these ECM components.

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NERVE GROWTH FACTOR (NGF) ENHANCES SURVIVAL OF HUMAN MELANOCYTES.

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During embryogenesis and after injury to the nervous system NGF is known to function as a survival factor for neurons. Because melanocytes (Mc) are known to express functional NGF receptors and keratinocytes are a potential local source of NGF in the epidermis, we asked whether NGF might enhance Mc survival after injury due to ultraviolet (UV) exposure or growth factor deprivation. Cultured human Mc were exposed to a solar simultor (5,10, 25 mj/cm2 UVB dose) or sham irradiated and then maintained in suboptimal serum-free medium, and continuously provided either with 50 ng/ml NGF or diluent alone and cell yields were determined. Both UV and suboptimal culture conditions reduced control Mc yields to below seeding at days 2 to 16 by up to 95% at day 16. However, in NGF-treated irradiated cultures Mc yields were up to 6.5 fold higher, with the greatest enhancement of survival at the most injurious UV dose. Mc yields in cultures maintained up to 16 days in suboptimal medium without UV were also enhanced by NGF, but less dramatically. To explore the mechanism of the striking response of UV irradiated cells to NGF, paired cultures were irradiated with 10 mj/cm2 UVB or were sham irradiated, and then incubated with antibodies to the high affinity component of NGF-receptor, trk. Mc in UV-treated cultures displayed more trk receptors than sham irradiated controls. Northen blot analysis checking the mRNA levels of the p75, the other component of NGF-receptor, showed several fold higher transcript levels in NGF-supplemented Mc than in diluent controls. Our data suggest that UV induces NGF receptors in Mc and that UV induced NGF of keratinocyte origin may enhance this up-regulation. We hypothesize a major role for NGF in Mc survival in habitually sun exposed skin, analogous to its role in the nervous system.

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ULTRAVIOLET B RADIATION INDUCES INCREASED PROLIFERATION AND MELANIZATION OF AGED MELANOCYTES COMPARED WITH YOUNG CELLS.

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With aging, sun exposure induces hyperpigmented lesion such as solar lentigine. To examine the effect of aging and ultraviolet radiation on growth, configuration and proliferation of melanocytes, we established normal melanocyte cell strains from foreskins aged 4 to 52 years. The doubling time of melanocytes of older than 20 years is twice longer than that of 4 years of age. The growth speed of melanocytes established from 52-year-old male decreased markedly after 3 months culture, while melanocytes of 4-year-old boy continue to grow stably more than 6 months. With aging in vivo, melanocytes have more dendrites, larger cytoplasm and darker pigmentaion. These characteristics were also observed after long cultivation of 4-year-old melanocytes in vitro, indicating that characteristics of aged melanocytes are inducible after certain cell divisions. By 2.5 mJ/cm^2 UVB, ^{3}H-thymidine uptake and pigmentation increased in aged melanocytes. These results indicate that aged skin has a potency of hyperpigmentation by UVB radiation, through efficient melanocyte division and melanogenesis.

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THE INCIDENCE OF NUCLEAR CAPS IN DYSCHROMIA.

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Nuclear caps are areas of melanin seen above the nucleus of basal cells. It is thought that they are derived from the phagocytosis of melanosomes or melanin granules, and they may have a function of providing some protection to the nucleus from UV irradiation.

We have developed a new technique for the analysis of melanin content of skin and its morphology in surface corneocytes by examination of tape strips from the skin surface. Using a haematoxylin and silver nitrate staining method with image analysis we have detected melanin in surface corneocytes in a similar distribution to that observed as nuclear caps in basal keratinocytes.

In a group of 24 normal volunteers we found little evidence of nuclear caps in corneocytes from unexposed sites, and a small incidence in more exposed areas. In a group of 20 patients with senile lentigo and melanocytic naevi, we found a very high incidence of nuclear cap-like melanin distribution in surface corneocytes from these lesions. In 5 patients with vitiligo we found not only no nuclear cap-like structures, but no melanin whatsoever (Table 1).

From the data obtained, it could be that nuclear cap structures in corneocytes reflect an abnormality in melanocytes at the sites examined, and their presence may be useful in characterising pigmentary anomalies.

Table 1: Incidence of nuclear cap-like melanin distribution in surface corneocytes per 1000 corneocytes.

Vitiligo	Lentigo	Normal exposed	Normal non-exposed
0.0	39.6 ± 16.7	7.5 ± 11.0	0.8 ± 2.1

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CHANGES IN MELANIN CONTENT OF SKIN AFTER UV IRRADIATION.

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Pigmentary changes in skin after ultraviolet irradiation are often used to evaluate photobiological responses. The time course of the pigmentary response is essential for the construction of dose-response curves, and for choosing an appropriate time for a single or an end-point measurement.

We have measured the time course of pigmentation after UV exposure using four methods(see table below). A specially constructed diffuse reflection spectrophotometer measures objectively an Optical Melanin Index(OMI). Image analysis of tape strips from the skin surface is used to measure the percentage area of surface corneocytes occupied by melanin granules and yields a Surface Melanin Index(SMI). Counting the number of DOPA positive melanocytes(those actively synthesising melanin) as a percentage of basal cells gives us our Histological Melanin Index (HMI). The 4th measure is the number of basal keratinocytes containing areas of melanin above the nucleus(nuclear caps) per 1000 basal cells (NC).

Day:	1	7	10	21	35
SMI	103 ± 26	136 ± 28	121 ± 19	551 ± 376	187 ± 75
HMI	71 ± 21	213 ± 94	229 ± 76	217 ± 43	216 ± 26
OMI	43 ± 31	111 ± 17	119 ± 21	123 ± 8	118 ± 18
NC	0	1.4 ± 3.1	1.6 ± 3.6	3.8 ± 1.3	1.2 ± 1.8

The reduction in OMI and HMI reflect the acute effects of UV damage to the melanocytes, which recover and respond by day 10. The surface melanin content shows that it takes 14-21 days for the changes in the basal layer to appear at the surface of the stratum corneum. The melanin meter seems to reflect the DOPA positive melanocyte response.

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DIFFERENTIATION OF MELANOCYTES AND EXPRESSION OF C-KIT IN THE CULTURED NEURAL CREST CELLS. Mitsuhiro Sato[1], Yoko Kawa[1], Yasuo Kubota[1], Masako Mizoguchi[1], Hirotake Ono[2], [1]Dept.of Dermatology, St. Marianna Univ. School of Med. Kawsaki, [2]Biological Institute, Faculty of Science, Tohoku Univ. Sendai, Japan.

To study a role of c-kit in melanocyte differentiation, primary neural crest cell cultures were prepared from E9.5 C57BL/6 mouse embryos, and c-kit expression on the cultured cells was immunohistochemically examined by using a monoclonal anti-c-kit antibody (ACK2, Nishikawa et al 1991) at cultured day 3, 6, 9, 15. One neural tube was placed on a dish and cultured in MEM medium with or without TPA and cholera toxin. The c-kit expression was noted on day 3 and was mainly induced by TPA, and assisted by cholera toxin. From thier dendritic appearance of c-kit positive cells, it was assumed that most of the positive cells might be melanocytes. Most of these cells became dopa-positive on day 9. Namely, melanocytes express c-kit on their cell surface before they differentiated into dopa-positive cells. Therefore, it is suggested that c-kit expression may play a role in melanocytes differentiation.

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CHANGE IN MELANOCYTES OF NEWBORN MICE BY ADMINISTRATION OF A MONOCLONAL ANTI-C-KIT ANTIBODY (ACK2). Mitsuhiro Okura, Masako Mizoguchi. Department of Dermatology, St. Marianna University School of Medicine, Kawasaki, Japan

We examined the development of melanocytes in newborn mice hair follicles by peritoneal injection of a monoclonal anti-c-kit antibody (ACK2). Melanocytes in the follicles, epidermis, and dermis were studied by light and electron microscopic examinations, dopa reaction, electron microscopic dopa and immunohistochemical examinations before and after ACK2 (kindly presented by Prof. Nishikawa, Kumamoto University) injection on days 0, 2, 4, 6, 8 post-partum. In normal untreated mice, melanocytes become active in the epidermis after birth and the number of dopa-positive melanocytes has increased up to day 4 post-partum. They gradually migrate from the epidermis to the hair follicles according to the development of the follicles. Thus, the number of epidermal melanocytes decrease after day 4 post-partum. In ACK2 treated mice, the earlier the mice received the ACK2 injection after birth, the fewer melanocytes they had not only in the epidermis, but also in their follicles. Destruction of dermal melanocytes on day 2 post-partum and the destruction of hair follicle melanocytes on day 3 post-partum were ultramicroscopically detected. Decrease in the number of dopa-positive melanocytes of hair follicles and epidermis obtained from mice administerted with ACK2 immediately after birth, was most significant. We thus assume that in newborn mice, the period of melanocytes' c-kit dependency is when melanocytes become active in the epidermis and migrate from the epidermis to the hair follicles.

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INHIBITORY EFFECT OF ESSENTIAL FATTY ACIDS ON MELANOGENESIS IN VITRO AND IN VIVO. Hideya Ando, Atsuko Ryu, Akira Hashimoto, Taketoshi Makino and * Masamitsu Ichihashi, Skin Care R&D Division, SUNSTAR INC., Osaka, Japan; * Department of Dermatology, Kobe University School of Medicine, Kobe, Japan

The purpose of this study was to evaluate the inhibitory effect of essential fatty acids, such as oleic acid (C18:1n-9), linoleic acid (C18:2n-6), and alpha-linolenic acid (C18:3n-3), on melanogenesis both in vitro and in vivo. In vitro cultured B16 mouse melanoma cells were treated with essential fatty acids (25 μM) for 3 days, and then the cellular melanin content and tyrosinase activity were determined. For in vivo experiment, UVB-induced pigmentation was established on the back of brownish guinea pigs, and the pigment decrease was measured after daily application of essential fatty acids (0.5% w/v) for 3 weeks. In addition, the alteration of skin turnover induced by essential fatty acids in albino guinea pigs was evaluated using dansyl chloride. The melanogenesis of B16 cells in vitro was inhibited most effectively by the treatment with alpha-linolenic acid, followed by linoleic acid and oleic acid. In contrast, the pigment decrease in vivo was accelerated more rapidly by the treatment with linoleic acid rather than by alpha-linolenic acid. Further, linoleic acid accelerated skin turnover more effectively than alpha-linolenic acid. These results suggest that the efficient effect of linoleic acid on the pigment decrease in vivo could be, in part, due to the acceleration of skin turnover rate, and also these essential fatty acids could be useful inhibitors of melanogenesis for medical use.

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ULTRASTRUCTURAL LOCALIZATION OF HMB-45 ANTIGEN IN THE FETAL MELANOCYTES AND MELANOMA CELLS. Arata Kikuchi, Hiroshi Shimizu and Takeji Nishikawa, Department of Dermatology, Keio University School of Medicine, Tokyo, Japan

HMB-45, a monoclonal antibody which recognizes a common antigen in the cytoplasm of melanoma cells and developing melanocytes but not in adult melanocytes, has been developed. HMB-45 antigen has recently been shown as an oncofetal protein of 10 kD, which appears in the cytoplasm of immature melanocytes in the skin around 50 days estimated gestational age. Ultrastructural localization of HMB-45 antigen has recently been detected in only melanoma tissues (stage I and II melanosomes) but not in the melanocytes of the fetal skin. In order to elucidate the precise ultrastructural localization of HMB-45 antigen, we examined fetal melanocytes from five abortus, melanoma tissues with or without melanin production and two types of cultured melanoma cells (G-361, Mewo) by using post-embedding immunogold electron microscopy with rapid freezing and freeze substitution fixation method without chemical fixatives. Our results revealed that HMB-45 antigen localized in all stages of melanosomes (stage I～IV) in the fetal melanocytes but in only stage I and II melanosomes in melanoma tissues and cultured melanoma cells. The reason for this discrepancy of HMB-45 antigen localization is not yet clarified, but it is possible that the level of maturation in melanogenesis is different between melanoma cells and fetal melanocytes, both of which are equally positive for HMB-45 in their cytoplasm in light microscopic immunohistochemistry.

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ANALYSIS OF THE PHOSPHORYLATION IN CULTURED HUMAN MELANOCYTES IRRADIATED BY ULTRAVIOLET RAY. Shih-Tsung Cheng, Chun-Ping Mak, Julia Yu-Yun Lee, Bei-Chang Yang*, Departments of Dermatology and Microbiology*, National Cheng-Kung University, Tainan, Taiwan, R.O.C.

Ultraviolet radiation (UVR) has been shown to act as the mitogenic and melanogenic stimulus for human melanocytes. The mechanisms are unknown. Disturbed growth factor networks have been suggested including abnormal induction of transforming growth factor-α, epidermal growth factor and receptor, and nerve growth factor receptor. Since these receptors have intrinsic protein kinase activity specific for tyrosine residues, it is to suspect phosphorylation in post-irradiated cells may give a distinct feature resulted from altered expression of growth factor receptors. In addition, phosphorylation of c-*Jun* protein is also activated by UVR possibly through Src tyrosine kinase activation.To study the effects of UVR on human melanocytes, we characterize the protein phosphorylation in melanocytes after UVR by radioactive labeling in vivo with [^{32}P]-orthophosphate and in vitro with [γ-^{32}P]-ATP. Several protein strongly labeled in vivo were labeled slowly or not at all in vitro. Those phosphoproteins, labeled to a varying extent, had been characterized by its molecular mass and isoelectric point. The phosphorylated ser/thr residues on these phosphoproteins were examined by alkaline hydrolysis. By incubation with [γ-^{32}P]-ATP, cell extracts catalyze the phosphorylation of at least 5 endogenous proteins, among them 2 are heavily labeled. The results suggest UVR may alter the activity of cytosolic protein kinases and/or phosphatases.

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PROLIFERATIVE ACTIVITY IN CUTANEOUS MELANOCYTIC TUMORS: DETERMINATION BY THREE DIFFERENT METHODS. I.Botev, L. Niteva, V.Nitev*, I.Todorov+ and R.Philipova+, Dept.of Dermatology, *Dept. of Biochemistry, Medical Faculty, +Inst.of Cell Biology, Bulg.Acad.Sci., Sofia, Bulgaria

The proliferative activity of melanocytic tumors has been studied intensively because the proliferating fraction may provide valuable information concerning cellular growth kinetics, tumor progression, and prognosis. The aim of the study was to evaluate the degree of "proliferative activity" in skin melanocytic lesions using 3 different methods. Argirophil technique for staining the nucleolar organizer regions (NORs) and two-step immuno-peroxidase method with a monoclonal antibody against 125 kD/pI 6.5 PCNA/mitotin were applied on a variety of 40 melanocytic formalin-fixed, paraffin-embedded specimens. Caseine kinase II (CKII) activity, after Mono Q column, was monitored with [γ-^{32}P]GTP and its specific substrate RRREEETEEE; spermine, polylysine, heparin, poly(Glu-Tyr)4:1, quercetin, and 2,3-bisphosphoglycerate were used for identification. The results showed a significant difference between the numbers of NORs per cell in benign and malignant lesions as a group, but some overlapping counts were found. Mitotin was expressed in significantly higher degree in metastatic and primary melanomas compared to common and dysplastic nevi. CKII activities from melanomas and dermal nevi were 5.9 and 2.5 fold higher than the skin.

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PRE- AND POST-TRANSLATIONAL EXPRESSION OF UV-INDUCED MELANOGENESIS IN MELANOMA CELLS. Hiroyuki Hara*, Takafumi Morishima*, Hua Chen, Dong Luo and Kowichi Jimbow, Department of Dermatology, Nihon University School of Medicine, Tokyo, Japan*, Department and Cut. Sciences, University of Alberta, Alberta, Canada

How sunlight affects the proliferation and differentiation of melanocytes is still unsettled. This study examined the nature of the cell activation and differentiation of melanocytes after UVB exposure. Human melanoma cells of SK-MEL 23 (highly pigmented), G361 (lightly pigmented), C32 and SK-MEL 24 (non-pigmented) were exposed to UVB at doses of 0-5 mJ/cm^2 for 7 consecutive days, and various aspects of melanogenic activities, e.g., melanin content, tyrosinase activity, immunoprecipitation of HMSA-5 (identical to b-locus protein, gp75), HMSA-6 (pre-Stage I melanosome) and HMSA-7 (lysosome protein) and mRNA expression of human tyrosinase and gp75 by polymerase chain reaction were evaluated. We foung, after exposure of UVB, that: (a) melanin content and tyrosinase activity were increased in pigmented cells but none of non-pigmented cells, (b) HNSA-5 expression was increased in melanotic cells but not in amelanotic cells, (c) HMSA-6 expression was also increased in both melanotic and amelanotic cells, and (d) tyrosinase and gp75 mRNAs were increased in melanotic cells. Importantly, while gp75 mRNA was not expressed in both C32 and SK-MEL 24, tyrosinase mRNA expression was seen in C32 but not SK-MEL 24. Our results indicate that a low dose of UVB stimulates melanocytes to initiate new melanogenesis at both pre- and post-translational levels, and that mRNAs of gp75 and tyrosinase are differently expressed (or activated) after UVB exposure and that HMSA-6 in not directly related to new synthesis of melanin and tyrosinase.

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EXPRESSION OF BASIC FIBROBLAST GROWTH FACTOR IN NEVUS CELL NEVUS AND MELANOMA. Masato Ueda, Keisuke Nishino, Yoko Funasaka, Masamitsu Ichihashi and Yutaka Mishima, Department of Dermatology, Kobe University School of Medicine, Kobe, Japan

It has been proposed that bFGF is an autocrine growth factor of melanoma cells in contrast to normal melanocytes where bFGF acts as a paracrine growth factor. Since this idea is mostly based on the different requirements of bFGF in culturing benign and malignant pigment cells in vitro, we performed immunohistochemical analysis to examine bFGF expression in vivo, using paraffin sections from nevus cell nevus (NCN) and malignant melanoma (MM). All of the NCNs (7 cases) showed strong and homogeneous expression of bFGF protein, whereas the primary MMs (5 cases) showed heterogeneous expression with the population of negative cells. Metastatic MMs (5 cases) were also heterogeneous with the increased population of negative cells. These results suggest that bFGF has some, but not identified, role in the growth of benign NCN and that the overexpression of bFGF is neither a prerequisite for the melanoma-genesis nor for the progression to metastatic MM.

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UVR-INDUCED DAG TURNOVER IN B16 MELANOMA CELLS AND SWISS 3T3 FIBROBLASTS. C.J.Carsberg and P.S.Friedmann. Department of Dermatology, University of Liverpool, U.K.

There is evidence that 1,2-diacylglycerols (DAGs) may mediate ultraviolet light (UVR)-induced melanogenesis in pigment cells. DAGs are mainly derived by receptor-mediated phospholipase cleavage of two cell membrane phospholipid species: phosphatidyl inositol (PIP_2) and phosphatidyl choline (PC). DAGs contain 2 fatty acid side chains. PIP_2-derived DAGs contain arachidonate (20:4) and either palmitate (16:0) or stearate (18:0) resulting in a total of 4 double bonds. PC-derived DAGs contain permutations of palmitate (16:0), oleate (18:1) and linoleate (18:2) resulting in a total of 0,1 or 2 double bonds. In this study we have measured the turnover of DAGs in response to UVR in B16 mouse melanoma cells and Swiss 3T3 mouse fibroblasts. We constructed time courses of total DAG production and separated the different types of DAGs, according to the number of double bonds, at each time point.

The cells were grown in culture dishes and exposed to a dose of UVR known to result in a doubling in melanin content of B16 cells. At each time point the cell lipids were extracted and DAGs were measured according to the methods of Preiss et al. (1) and Kennerly (2).

In both cell types a biphasic time course was seen. In B16 cells peaks were seen at 30 seconds (198% of resting levels) and 30 minutes (237%) and the rises were mainly due to increases in the 0 and 1 double bond DAGs. In 3T3 cells the peaks occured after 1 minute (132%) and 15 minutes (148%) and consisted mainly of 0,1 and 4 bond DAGs.

These results show that UVR causes DAG turnover in both cells types and that PC is the main source in B16 melanoma cells whereas PC and PIP_2 are the predominant sources in 3T3 cells.

The data from this study is further evidence of a role for DAGs in UVR-mediated melanogenesis. Since the response appears non-specific, a distinguishing step further along the melanogenic pathway is expected.

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THE PRESENCE OF ESTROGEN RECEPTOR AND ESTROGEN RESPONSING ON NORMAL HUMAN MELANOCYTES. Shiou-Hwa Jee, Sheng-Yih Lee, Tong-Jen Chen, Department of Dermatology, College of Medicine, National Taiwan University, Taipei, Taiwan.

The reports about the presence of estrogen receptors of malignant melanoma and of dysplastic nevi were contradictory. The reason may be the individual difference, difference in differentiation and the various methods used to detect estrogen receptors. The tritiated estrogen used in biochemical method may be hydroxylated by tyrosinase to release tritiated water. For avoiding the miss counting to tritiated water as estrogen receptor, we used hydroxyapatite column assay in stead of dextran-coated charcoal method to determine the estrogen receptor on normal human melanocytes in vitro. The presence of estrogen receptor (59.13±17.12 fmole/mg nuclear protein and 5.42±1.11 fmole/mg cytosol protein) was found. Meanwhile, the cell number increased and the melanin decreased in a dose response pattern when 17-β-estradiol interacted with melanocytes at the physiological concentration of 0, 10^{-12} and 10^{-9}M in vitro.

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MELANOGENESIS DURING THE ANAGEN-CATAGEN-TELOGEN TRANSITION OF THE MURINE HAIR CYCLE. Andrzej Slominski[1], Przemyslaw Plonka[2], Ashok Chakraborty[3], Stanislaw Lukiewicz[2], Beate M Czarnetzki[4] and Ralf Paus[4]. [1]Department of Microbiology, Immunology and Molecular Genetics, Albany Medical College, Albany, NY 12208, USA, [2]Institute of Molecular Biology, Jagiellonian University, Krakow, Poland, [3]Department of Dermatology, Yale University School of Medicine, New Haven, CT, USA and [4]Department of Dermatology, UKRV, Freie University Berlin, Berlin, Germany.

In mice, the melanogenic activity of follicular melanocytes is strictly coupled to the anagen stage of the hair cycle. In catagen, melanin formation is switched-off and is absent throughout telogen. We have followed the expression and activity of melanogenesis related proteins (tyrosinase and DOPAchrome tautomerase) in relation to melanin synthesis during the anagen-catagen-telogen transition of the depilation-induced hair cycle of C57 BL-6 mice. The disappearance of melanogenically active follicular melanocytes at the very end of anagen VI was accompanied by a relative decrease in the melanin content in the full-thickness skin as measured by EPR spectroscopy. However, a rapid decrease in tyrosinase and DOPAchrome tautomerase activities and in tyrosinase concentration occurred only 2 days later, i.e. during catagen, which most likely reflected the presence of catalytically active and immunodetectable enzymes in melanosomes already transferred into follicular keratinocytes during anagen VI.

We suggest that the rapid decrease in the concentration and activity of tyrosinase and DOPAchrome tautomerase are good and novel biochemical markers of catagen induction, but do not reflect melanogenic activity of follicular melanocytes in this complex tissue interaction system.

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PIGMENT PRODUCTION IN MURINE MELANOMA CELL IS REGULATED BY TYROSINASE, TYROSINASE-RELATED PROTEIN 1 (TRP1), DOPACHROME TAUTOMERASE(TRP2), AND A MELANOGENIC INHIBITOR. Koichiro Kameyama, Chie Sakai, Tosiyuki Takemura, Shigeo Kondoh, Kazunori Urabe and Vincent J. Hearing. Department of Dermatology, The Kitasato Institute Medical Center Hospital, Saitama, Japan, Laboratory of Cell Biology, National Institutes of Health, Bethesda, MD, USA.

Using antibodies that recognize either tyrosinase, tyrosinase-related protein-1 (TRP1), or tyrosinase-related protein-2 (TRP2, DOPAchrome tautomerase), the quantities of those melanogenic enzymes were analyzed in five melanoma cell lines that possess various degree of melanin production. There was a positive correlation between quantities and synthetic rates of those melanogenic enzymes and their melanin formation or DOPAchrome tautomerase activities. The activity of a heat-resistant melanogenic inhibitory factor was also analyzed. The results showed, surprisingly, that pigmented cells showed higher levels of melanogenic inhibitor activity. Tyrosinase activity was increased dramatically whereas the level of melanogenic inhibitor was remarkably decreased following MSH treatment. Interestingly, melanogenic inhibitor derived from JB/MS-W cells suppressed not only tyrosinase but also DOPAchrome tautomerase, another enzyme functional in melanin production. These results clearly suggest that melanin production is regulated by a subtle balance between the activities of these enzymes and other factors such as the melanogenic inhibitor.

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A COMPARATIVE STUDY ON THE PATHOGENESIS BETWEEN SEGMENTAL AND NONSEGMENTAL TYPE VITILIGO. Kao Chao-Hsing, Yu Hsin-Su ,Department of Dermatology, Kaohsiung Medical College, Kaohsiung, Taiwan, ROC.

Vitiligo vulgaris has been classified into segmental and nonsegmental types for a long time. However, there is no definite evidences to support the hypothesis that they are two different diseases. In this study, we evaluate the presence of antimelanocyte antibodies in the sera of both type vitiligo patients, and also the cutaneous physiological examination, such as skin electric current(SEC). The results revealed that none of the sera of patients with segmental type vitiligo revealed the presence of antimelanocytes antibodies(AMA). However AMA was found to present in the sera of about 33% patients with nonsegmental type vitiligo. In patients with active segmetnal type vitiligo (i.e. new depigmented lesions observed within recent 3 months), the SEC was significantly different between the depigmented lesions and symmetrically opposite normal skin. However, there was no difference of SEC between the depigmented lesions and symmetrically opposite normal skin in nonsegmental type vitiligo. In conclusion, it is proposed that the dysfunction of autonomic nervous system in certain dermatome may result in segmental type vitiligo, and immuno-logical dysfunction may paly a role in the pathogenesis of nonsegmental type vitiligo.

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EPICAN, A HEPARAN SULFATE PROTEOGLYCAN FORM OF CD44 ON KERATINOCYTES, IS A CELL-CELL ADHESION MOLECULE. Leonard Milstone, Lisa Kugelman and John Haggerty, Department of Dermatology, Yale University School of Medicine, New Haven, CT, USA.

Proteoglycans have a variety of adhesive functions, and cell-cell adhesive interactions play a central role in tissue morphogenesis and tumor biology. The goal of our work has been the molecular characterization of cell surface proteoglycans on keratinocytes and the identification of their function. Most keratinocyte proteoglycans are large, predominantly heparan sulfate proteoglycans. The core protein of one heparan sulfate proteoglycan was identified using a monoclonal antibody which we raised against a preparation of keratinocyte proteoglycans. The epidermal intercellular proteoglycan (epican) is expressed on the surface of keratinocytes and a limited number of other epithelial cells but is not detected at the basement membrane zone nor on most mesenchymal cells. The cDNA-derived sequence for the core protein of epican proved to be a newly recognized, alternatively-spliced protein variant of CD44, a transmembrane protein that interacts with the cytoskeleton and extra-cellular matrix. Epican is the longest protein variant of CD44 identified to date and contains all but one of the protein coding exons. Keratinocyte-keratinocyte adhesion is strongly inhibited by an antibody to the core protein of epican. Transfection of the epican cDNA into mouse L cells results in the expression of a new cell surface proteoglycan and greatly increased adhesion of transfected cells to keratinocytes.

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INTERLEUKIN-1-BETA AND TUMOR NECROSIS FACTOR-ALPHA INCREASE LYMPHOCYTE AND NEUTROPHIL CHEMOTACTIC ACTIVITY OF HUMAN MELANOMA CELL CULTURE SUPERNATANTS. Kazufumi Yoneda and Shunji Mori, Department of Dermatology, Gifu University School of Medicine, Gifu, Japan

Many cytokines have been found in the supernatant of cultured melanoma cells, such as interleukin-1(IL-1), IL-3, IL-8 and tumor necrosis factor-alpha(TNF-alpha). These factors might attract inflammatory infiltrate around melanoma cells. In this study, we investigated effects of different cytokines on lymphocyte and neutrophil chemotactic activity of cultured human melanoma supernatants. In addition, we assayed the concentration of IL-8 in the supernatants. Lymphocytes and neutrophils were enriched from normal human peripheral blood by Mono-poly resolving medium(ICN Biochemicals). Supernatants of cultured human melanoma cells stimulated with or without interferon-alpha(IFN-alpha), IFN-beta, IFN-gamma, IL-1-beta, IL-2 or TNF-alph were assayed for both chemotaxis and IL-8. Chemotaxicell(Kurabou) of 5 micron and 3 micron in diameter were employed for lymphocyte and neutrophil chemotaxis assay, respectively. Quantikine human IL-8 immunoassay kit(R&D systems) was employed for IL-8 assay. Human melanoma cell culture supernatant showed different degree of lymphocyte and neutrophil chemotactic activity. Among the cytokines used in the experiment, only IL-1 and TNF-alpha increased chemotactic activity of melanoma supernatant. Anti-IL-8 antibody inhibited the increase chemotactic activity of the supernatants. IL-8 concentration in the melanoma supernatants increased by the stimulation of either IL-1 or TNF-alpha. In conclusion, IL-1beta and TNF-alpha increased chemotactic activity of human melanoma cell culture supernatants. IL-8 probably play major role in the increased chemotactic activity.

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INFLUENCE OF CYTOCHALASIN A ON SINGLE CELL MOTILITY OF MELANOMA CELLS IN VITRO. Rainer Hofmann-Wellenhof, Josef Smolle, Regina Fink-Puches, Christine Helige, Helmut Kerl, Department of Dermatology, University of Graz, Austria

Cell motility is a crucial property of tumor cell invasion and metastasis. We developed an automated system to measure the translocation and membrane ruffling of single cells during movement. In this study we investigated the influence of cytochalasin A, which is known to prevent elongation of actin filaments on single cell motility of K1735-M2 mouse melanoma cells.

The cells were seeded at low density into a microincubator. Time lapse microcinematography was performed every 20 seconds from a high power field to assess membrane ruffling and every 10 minutes with a screening objective to measure translocation. In the second step of the investigation cytochalasin A 1 ymol/l was added to the medium. For calculation of membrane ruffling each image was subtracted from the next following and the resulting image, reflecting the changes having taken place in the time interval, was used for quantitative evaluation. We calculated the area of change (AC) and the density change (DC) given in pixels per cell. To measure the translocation the center of gravity of each cell was assessed subsequently and the velocity was calculated by connecting the centers of gravity.

The cytochalasin A treated cells showed a significant lower AC and DC ($AC=0.008,\pm0.015$; $DC=32.3,\pm12.07$; U-test:$p<=0.01$) compared to the untreated cells ($AC=0.045,\pm0.022$; $DC=71.5,\pm27.8$). Additionally, the velocity of cells grown with cytochalasin A (11.56 ym/h, ±14.68) was significantly lower than that of the control group (37.96 ym/h, ±14.6).

In conclusion our study shows that our system enables observation and quantitative assessment of the different expressions of single cell motility in vitro. Cytochalasin A inhibits significantly both stationary and translocative single cell motility of melanoma cells.

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EOSINOPHIL PEROXIDASE IS SUPERIOR TO MAJOR BASIC PROTEIN AS A MARKER FOR EOSINOPHIL IN HUMAN SKIN. Binghe Wang, Dermatology Research Laboratory, Nanjing Medical College, Nanjing, P.R.China

Eosinophil peroxidase (EP) is a 78 kd enzyme uniquely associated with human eosinophils. In this study, we used EP as a maker for the identification of eosinophils among the infiltrating cells in atopic dermatitis (AD) lesional skin and made a comparison between EP and major basic protein (MBP).

Skin cryostat sections from 15 patients with AD, and 10 healthy individuals as control, were labeled with an anti–EP mAb (Oncogen science, NY) or anti–MBP mAb by Indirect Immuno–Fluorescence or ABC technique. We found that EP^+ cells were present among the cutaneous infiltrates in all the 15 AD patients. The number of EP^+ cells varied from patients to patients, ranging from a small population to 10% of the infiltrating cells. By contrast with the anti–EP labeling, few MBP^+ cells except some MBP^+ eosinophil remnants were observed in the infiltrates in all the samples examined.

These results demonstrate that EP is superior to MBP as a marker for eosinophils in human skin.

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FURTHER STUDIES ON THE ROLE OF PROTEIN KINASE C IN Fc_ϵRI-MEDIATED EXOCYTOSIS AND EICOSANOID PRODUCTION FROM HUMAN BASOPHILS. Ulrich Amon, Esther von Stebut, Urda von Gyzicky and Helmut H. Wolff, Department of Dermatology, University of Lübeck, Lübeck, Germany

IgE-mediated hyperreleasability from atopic basophils (B) is accompanied by a decreased in vitro response towards activators of protein kinase C (PKC) suggesting a control function of PKC for Fc_ϵRI-transduced exocytosis [1]. The present study investigated the effects of novel selective PKC inhibitors on the Fc_ϵRI-mediated activation of B. Cells were purified from the peripheral blood by dextran-sedimentation, gradient centrifugation and incubation with CD2+ and CD19+ immunobeads to remove T- and B-cells. Purity of B was ca. 80%. The selective PKC inhibitors Ro 31-7549, GF 109203X, and calphostin C produced a dose-dependent potentiation of histamine release induced by anti-IgE antibodies, indicating again a negative regulatory function of PKC for this pathway. However, preincubation of B with low concentrations PKC activators (different phorbol esters) did not inhibit the Fc_ϵRI-mediated response. Down regulation of PKC by long term exposure to phorbol ester PMA (20nM, 6h) reduced the PMA-induced exocytosis whereas the IgE-mediated response was only reduced by 30% vs. control. This provides further evidence that phorbol ester-induced and IgE-mediated exocytosis are transduced by distinct PKC isozymes [2]. In contrast to anti-IgE antibodies, different activators of PKC (phorbol esters and diacylglycerol derivatives) did not induce production of LTC_4 from B. However, LTC_4 release was significantly enhanced by incubation with selective PKC inhibitors, suggesting a link between PKC activation and LTC_4 production. [1] Amon U *et al.* Dermatology 186: 109; 1993 [2] Baeven M *et al.* J. Biol. Chem. 268: 1749; 1993

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ESTABLISHMENT AND CHARACTERIZATION OF A CELL LINE DERIVED FROM MALIGNANT GRANULAR CELL TUMOR. Shinichiro Yasumoto, Atsumichi Urabe, Akito Toshitani, Shuhei Imayama and Yoshiaki Hori, Department of Dermatology, Faculty of Medicine, Kyushu University, Fukuoka 812 and Kyushu Koseinennkin Hospital, Kitakyushu 806

We have estabished a cell line from tissue specimens of malignant granular cell tumor which had been implanted in nude mice. Initial attempts to culture tumor derived cells in RPMI 1640 medium supplemented with 10% fetal calf serum resulted in senescence of tumor derived cells and the dominance of the culture with murine fibroblasts. By a use of serum-free melanocyte growth medium, a cell line consisted mainly with tumor derived cells(MGCT-4) was established. Cultured cells were dendritic in appearance, positive for myelin basic protein in immunohistochemistry and lysozome-rich in electron micoscopic observation. These results suggest that the cell line is Schwann cell origin and may help further understanding of the biologcal nature of this tumor .

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THE CELLS FROM THE PATIENTS OF TUBEROUS SCLEROSIS LOST THE NUCLEAR ANTIGEN DETECTED BY ANTI-CHROMOSOMAL MONOCLONAL ANYIBODY M108 . Mari wataya-Kaneda, Yasufumi Kaneda, Koji Hashimoto and Kunihiko Yoshikawa, Department of Dermatology, Osaka University Faculty of Medicine, Osaka, Japan

Tuberous sclerosis is a multi-systemic disorder that is inherited as an autosomal dominant trait and is characterized by systemic hamartomas. Some of the cultured cells derived from lesions of tuberous sclerosis showed the distinct chromosomal disarrangement and abnormal cell division. To detect the proteins responsible for the abnormal cell division of tuberous sclerosis, we investigated many nuclear or chromosomal components using monoclonal antibodies. Immunohistochemically, we investigated the cells from 6 different patients and found that in all the patients 20 to 60 % of the cells lost the antigen recognized by an monoclonal antibody M108(Ab. M108). We previously reported that the 40 kDa protein detected by the Ab. M108 was present in the nuclear envelope, perichromosomal region and vesicle-like structure in the cytoplasm of many vertebrate culture cells. The ratio of the decrease of antigen detected by Ab. M108 was in propotion to the degree of disease severity. The patients with severer mental retardation and more facial angiofibromas had the less amount of the antigen recognized by Ab. M108. Furthermore immunoblotting using Ab. M108 also revealed the correlation between the symptoms of the disease and the loss of 40 kDa antigen. Thease results indicate that the nuclear antigen detected by Ab. M108 plays an important role in the pathogenesis of tuberous sclerosis.

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THE INFLUENCE OF DNA DAMAGING AGENTS ON THE INDUCTION OF 72KD HEAT SHOCK PROTEIN Haruhiko Ohno, Yukio Yamashina, Tsutomu Muramatsu, Nobuhiko Kobayashi, Masami Yamaji, Toshihiko Shirai and Hideki Matsumoto*, Department of Dermatology and First Department of Anatomy*, Nara Medical University, Kashihara, Japan

It is well known that various environmental stresses induce the synthesis of so-called stress proteins. We previously reported that the 72kD heat shock protein(HSP72) was induced in the cultured xeroderma pigmentosum group A fibroblasts(XP2OSSV) and normal fibroblasts(WI38VA13) by UVC irradiation. In the present study, we examined the inducibility of HSP72 by the exposure to various DNA damaging agents in the same cell lines by the immunofluorescence method using a monoclonal antibody specific for the HSP72. The induction of HSP72 were observed in both cell lines on dose dependency of 4NQO, CDDP, BLM, MMS, MMC, ACNU and MNNG. To the base damaging agents, XP2OSSV cells were more responsible than WI38VA13 cells. We consider that the induction of HSP72 by DNA damaging agents may relates to cell death, the disturbance of cell mitosis and/or protein synthesis. And there is a possibility that HSP72 plays a role in the DNA repair.

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DETECTION BY FLUORESCENCE IN SITU HYBRIDIZATION (FISH) OF TWO DIFFERENT MECHANISMS OF DNA DAMAGE IN MICRONUCLEI INDUCED BY IONIZING RADIATION AND COLCEMIDE IN A HUMAN EPIDERMAL CELL LINE. Andreas P.Rhein*,Klaus-Peter Gilbertz*, Ralf U.Peter*,** * Institute of Radiobiology, Federal Armed Forces Medical Academy, ** Department of Dermatology, Ludwig-Maximilians-University, Munich, Germany

The micronucleus frequency (MNF) is a well-established method to estimate the amount of DNA damage caused by ionizing and ultraviolet radiation, chemotherapeutic agents, carcinogens and others. Principally, two mechanisms of micronucleus (MN) formation, clastogen (generation of chromosome fragments) and aneugen (exclusion of total chromosomes), have been described. As formation of MN requires at least one cell division after exposure, alterations of cell cycle kinetics have a strong impact on appearance of MN. Therefore, as long as cell cycle kinetics or externally-induced alterations of these have not been determined, the assessment of MNF as a predictive assay for cellular DNA damage is of limited value. We analyzed MN formation in the human epidermal SCLII cell line after exposure to ionizing radiation (X-rays, 1-6 Gy, 240 kV, 15 mA) and Colcemide (300 ng / ml; 10 hrs exposure) . Cell cycle kinetics were assessed by flow cytometry after double labelling of DNA and incorporated BrdU. MNF in this moderately radiosensitive cell line (Do = 1,6 Gy; Dq = 1,6 Gy) was determined as up to 40%, depending on irradiation dose. Colcemid treatment resulted in a markedly lower MNF of only 8%. In both assays, MNF could be determined only after cells left the irradiation- or colcemide- induced G2 block. Characterization of MN with fluorescence in situ hybridization (FISH) using a centromer-specific DNA probe revealed up to 80% centromere positive MN in colcemide treated cells, in contrast to 90% centromere negative MN in the irradiated cells. These data demonstrate that in this experimental setting DNA damage is induced in two different ways, namely clastogen after ionizing radiation and aneugen after Colcemide. In addition the usefulness of this modified MN assay corrected for cell kinetics and closer characterized by FISH, in determining mutagenic or cytotoxic potential of various agents, which are of importance also in clinical dermatology, has been shown. Further studies will be directed towards detection of DNA sequences of growth regulating genes or oncogenes in MN after radiation exposure.

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STUDIES ON RADIOENHANCEMENT OF X-RAY INJURIES BY CAMPTOTHECIN-11 OR PEPLOMYCIN TO THE NORMAL SKIN. Kenichi Kawahara, Eiichi Kano * and Keiichi Ueda, Department of Dermatology, *Department of Experimental Radiology and Health Physics, Fukui Medical School, Fukui, Japan

X-ray injuries of the normal skin are chemically radioenhanced by various anticancer substances. Camptothecin-11(CPT-11), an inhibitor of DNA topoisomerse I, or peplomycin(PEP), a kind of anticancer antibiotics, were used to study the interactive effects of combined treatment with X-ray and these drugs. Gross changes occurring on the skin of ICR hairless mice after 10Gy of X-ray irradiation were scored and as the result of the scoring of skin injuries the intraperitoneal administration of PEP(30 mg/kg body weight) significantly radioenhanced X-ray injuries, while that of CPT-11(15 mg/kg body weight , within the clinical dose range and the same molarity as PEP) or CPT-11(50 mg/kg body weight) did not reveal skin injuries. Chemical radioenhancement ratio of the combined treatment with X-ray and PEP(30 mg/kg body weight), CPT-11(15 mg/kg body weight) or CPT-11(50 mg/kg body weight) were 1.49, 1.17 and 1.34, respectively.

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LESIONAL PSORIATIC KERATINOCYTES CAN BE CULTURED ON A PERMEABLE COLLAGEN MEMBRANE IN A NORMAL Ca^{2+} CONDITION. Y. Miyauchi, T. Kikuchi, Y. Mitsuhashi, and I. Hashimoto, Department of Dermatology, Hirosaki University Schoool of Medicine, Hirosaki, Japan

Lesional psoriatic keratinocytes (LPKs) have been considered to be hardly cultured in a normal calcium condition without specific proliferative agents, such as cholera toxin, and fibroblast-derivatives. By using a permeable collagen membrane, we succeeded in culture of LPKs in a normal Ca^{2+} (1.4mM) condition without any specific proliferatives. LPKs were obtained from chronic plaques of 3 patients with psoriasis vulgaris. The epidermis was separated with dispase. Single cell suspensions were prepared by trypsin treatment. The cells were cultured with Eagle's MEM containing antibiotics and 10% fetal calf serum. Proliferation of the cells on the collagen membrane was observed with a phase-contrast microscopy. The medium was harvested and renewed in every 4 days. Twelve hours after inoculation, LPKs attached to the membrane and they gradually proliferated. They reached confluent by the 10th day of culture. Using this culture system we studied secretions of TGFα, IL-6 and IL-8 in the medium of LPKs and compared them with those of normal keratinocytes (NKs). Significantly increased secretions of TGFα and IL-8 by LPKs were observed at the initial phase of culture but their secretions decreased during the time course. IL-6 was low in both media of NKs and LPKs even at the initial phase. Our results clearly demonstrated that LPKs secreted larger amounts of TGFα and IL-8 but not IL-6.

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ARE FIBROBLAST GROWTH FACTORS MAIN GROWTH FACTORS IN BOVINE PITUITARY EXTRACTS FOR THE CULTURED HAIR APPARATUS CELLS? Noriko Obana and *Masaaki Ito, Hair care R&D Division, Sunstar Inc., Osaka and *Department of Dermatology, Niigata University School of Medicine, Niigata, Japan

Acidic and basic fibroblast growth factors (aFGF and bFGF, respectively) are polypeptides that modulate functions of various cells and exist in a wide variety of tissue sources including bovine pituitary. In the present study, we examined the effects of aFGF and bFGF on proliferation and differentiation of the cultured hair apparatus cells. Further, whether both FGFs are main growth factors in bovine pituitary extracts (BPE) was also examined. The hair apparatus cells isolated from 4-day-old C3H mice were maintained in MCDB 153 either with aFGF, bFGF or BPE. Both FGFs and BPE stimulated cell proliferation and induced cell differentiation into several subpopulations corresponding to those of in vivo cell layers of the hair apparatus by electron microscopy. Heparin enhanced the stimulating activity of aFGF, while it unaffected that of bFGF. Further, heparin in part inhibited cell differentiation induced by aFGF. Although both aFGF- and bFGF-like proteins were detected in Western immunoblots of the BPE, BPE treated with anti-aFGF and/or anti-bFGF antibodies still showed a cell proliferating activity. These data suggest that both FGFs may be one of growth factors; however, some unknown growth factors other than FGFs, which show a great proliferating activity for the cultured hair apparatus cells, may exist in BPE.

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NODULAR BASAL CELL CARCINOMA CELL LINES: MORPHOLOGY, IMMUNOPHENOTYPE AND BIOSYNTHETIC ACTIVITY. OMV Schofield, DA Kist, APN Skubitz[1], CB Zachary, SA Grando Departments of Dermatology and [1]Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, MN, USA.

We have established immortal cell lines of basal cell carcinoma (BCC) and characterised their cell morphology, immunophenotype and biosynthetic activities. Contaminating fibroblasts and keratinocytes (KC) were removed from primary cell cultures by a brief exposure of the cultures to trypsin/EDTA and temporarily increasing the concentration of extracellular Ca^{2+} from 0.09 to 0.8 mM, respectively. The BCC cells in pure lines exhibit changeable morphology and formed colonies with pavement organization. The cells appear immortal as they continue dividing after ten months. They exhibit an immunophenotype distinct from normal KC (absent bullous pemphigoid and pemphigus antigens, epiligrin, $\alpha 6$ integrin; positive fibronectin and $\alpha 3$ integrin) and other dendritic cells (absent S100, HMB45, CDIa, HLA-DR, neuron specific enolase, Factors VIII and XIIIa) but similar to BCC in-vivo (positive keratins 5 and 14, as judged by immunohistochemistry and western blotting). Extracellular material containing keratin and filaggrin was also identified. The malignant nature of the established BCC cell lines was confirmed by staining of the skin tumor nests with antibodies raised in rabbits immunised with the cultured cells. In response to the differentiating agents phorbol-12-myristate-13-acetate, sodium butyrate and 1.6 mM Ca^{2+}, cultured tumor cells started synthesizing a 67kD keratin protein detected by immunoblotting.

Thus we have established nodular BCC cell lines and for the first time demonstrate their unique cytomorphology, behavior and antigenic phenotype.

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ESTABLISHMENT AND CHARACTERIZATION OF A MERKEL CELL CARCINOMA CELL LINE Tadashi Karashima, Hiroshi Hachisuka, Yoichiro Sasai, Department of Dermatology, Kurume University School of Medicine, Kurume, Japan

Merkel cell carcinoma is an uncommon carcinoma of the skin, and regarded as a neuroendocrine tumor. In this study, a Merkel cell carcinoma cell line has been established from a 32-year-old Japanese woman. A 0.5cm block, which was obtained from firm, solid tumor of the scalp, was mechanically dissociated, and the cells were cultured in D-MEM with 10% FCS and were passaged with 0.05% trypsin and 0.02% EDTA treatment. The cells adhered to the culture flask and extend broad cytoplasmic projections. A mean doubling time was 140 hours at the 23 passages. EGF induced the enhancement of the cell proliferation. The culture soup contained of high levels of NSE, serotonin. Cells had dense-cored granules (100nm in diameter) in the cytoplasm, and intermediate filaments. Poorly developed desmosomes were present. The cells were immunohistochemically positive for NSE, chromogranin A, and N-CAM. The cells possessed simple epithelic type keratin intermediate filaments, but no neurofilaments. In the karyotype analysis, chromosome alterations involving chromosome #1, 6, 7, 9, 11, 22, and unidentifiable marker chromosomes were observed.

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ESTABLISHMENT AND CHARACTERIZATION OF A MERKEL CELL CARCINOMA CELL LINE. Hikaru Eto, Kohzoh Yonemoto, Takao Fujimura, Yuko Hamada, Shigeo Nishiyama, Michiko Ohno* Tohru Maeda* and Tadao Funato**, Dept. of Dermatol., Kitasato Univ. Sch.of Med., *Cytogenet. Lab., Kitasato Univ. Hospital, Sagamihara, and **Dept. of Clin. and Lab. Med.,Sch. of Med.,Tohoku Univ. Sch. of Med., Sendai, Japan

A novel Merkel Cell Carcinoma cell line, KMCC2, has been established from a metastasized lymph node of 62-year-old Japanese female. Morphologically, the tumor cells were ovoid and relatively small in size and showed chain-like configuration which tend to form clusters at floating condition. The population doubling time was 42 hours. Ultrastructurally, incomplete desmosomes and small number of dense-core granules were observed. A chromosome analysis revealed hyperdiploidy ranging from 50-52 chromosomes with various numerical and structural aberrations. The cytokine mRNAs were analyzed by RT-PCR method, however, the messages of IL-1alpha/beta, IL-3, IL-6, IL-8, GM-CSF, TNF-alpha and TGF-beta were all negative. Neuron specific enolase (NSE) was positive in the cells by immunostaining and the culture supernatant contained 43.2ng/ml of NSE by EIA. This result correlated with original tumor which were NSE positive by immunostaining. Ras oncogene and p53 suppresser gene were also analyzed. In conclusion, our KMCC2 cell line would be an useful tool to further study the normal as well as neoplastic Merkel cells.

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INDUCTION OF TERMINAL DIFFERENTIATION OF SQUAMOUS CELL CARCINOMA BY BUFALIN. Youngsook Son[1], Kwang Hyun Cho[2], Myung Hee Chung[1], Chan Woong Park[1], Takemi Yoshida[3], and Yukio Kuroiwa[3]. [1]Department of Pharmacology and [2] Department of Dermatology, Seoul National University College of Medicine, Seoul, Korea[3] Department of Biochemical Toxicology, School of Pharmaceutical Sciences, Showa University, Tokyo, Japan

We investigated whether retinoic acid, Vit D_3, cAMP, sodium butyrate, DMSO, TPA, and bufalin, differentiation inducing agents in other tissues, can stimulate terminal differentiation of epidermis. Squamous cell carcinoma cell line (SCC 13) which is highly defective in terminal differentiation, was cultured at air-liquid interface(raft culture) in the presence of differentiation inducing agents alone or in combination. Among them, bufalin which was previously reported to induce differentiation of human leukemia cells, can induce terminal differentiation of SCC 13 raft culture at morphological and biochemical aspect. Bufalin at 10 nM stimulates expression of keratin 1, filaggrin, involucrin in the suprabasal layers of SCC 13 raft cultures but decrease the number of proliferating cells. This concentration of bufalin did not affect on cellular metabolic rate based on ^{35} S-methionine incorporation and MTT cytotoxicity assay. Mechanism underlying differentiation inducing ability of bufalin and its possible clinical application for psoriasis and skin cancer are studying. As one of them, bufalin specifically inhibits type 1 collagenase induction of SCC 13 cells mediated by TGF alpha.

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EFFECT OF CYCLOSPORIN, MINOXDILE, TESTOSTERONE, ESTRADIOL AND CORTICOSTEROID ON HAIR GROWTH OF NUDE MICE. Yutaka Hozumi, Tsutomu Imaizumi and Shigeo Kondo, Department of Dermatology, Ymamagata University Faculty of Medicine, Yamagata, Japan

Cyclosporin is now widely used as a potent immunosuppressive agent in organ transplantation. One of the effects of the drug is hypertrichosis. Similar hair growth has been repoted in nude mice and this is a good model to study the hair growth mechanism. We also investigated the effect of minoxidil, testosterone, estradiol and corticosteroid on the hair growth of nude mice. And we also evaluated effects of the combinations of cyclosporin and the other agents.

Aliquots of solutions of cyclosporin and other agents were applied once per every day topically on the tails and the lower backs of 5 week-old BALB/C nude mice, for as long as 6 weeks.

The stimulated hair growth on the mice treated with cyclosporin, minoxidil and testosterone was noticed in 5 or 7 days of application of the agents, but it stopped in a few days. The hair growth seemed to be influenced by the hair cycle of the nude mouse, being to be around 30 days. Combination of cyclosporin and other agents demonstrated that there was not additive nor synergistic effect, but other agents seemed to inhibit the cyclosporin effect.

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EXPLANT CULTURE OF OUTER ROOT SHEATH CELLS FROM HUMAN HAIR
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Keratinocytes from the outer root sheath of human follicles were successfully grown in vitro. Isolated hair follicles were treated by collagenase type IV at 37°C in an atmosphere of 5% CO2/air incubation for 70 minutes. The enzyme treated hair follicles were cultivated in media prepared by different combinations of growth factors in Eagle's Modified Essential Medium. The growth factors were hydrocortisone, insulin, fetal calf serum and epidermal growth factor.

Ca++ and cracking of the outer root sheath were necessary for migration of keratinocytes from the outer root sheath. Among the growth factors epidermal growth factor was the most important. Outer root sheath cells grew well and proliferated more than 2 months in serum free medium. New serum-free medium with epidermal growth factor was established for culture of the outer root sheath cells. Fetal calf serum contains various unknown factors, so it sometimes interrups the correct interpretation of the efficacy of other ingredients. Therefore, it is important to establish a serum-free medium for evaluating the influence of growth factors and studying of hair follicle biology.

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THE METABOLISM OF TESTOSTERONE BY DERMAL PAPILLA CELLS CULTURED FROM SCALP FOLLICLES OF MEN WITH ANDROGENETIC ALOPECIA.
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Androgens regulate much human hair growth and are implicated in many human hair growth disorders such as androgenetic alopecia and hirsutism, but the mechanism of androgen action in human hair follicles is not fully understood. Since androgens probably act on hair follicles via the dermal papilla (DP), cultured DP cells appear to offer a good model system. We have derived DP cells from scalp follicles of men with male pattern baldness and investigated their testosterone metabolism.

Primary DP cell lines were derived from balding (n=5) and nearly clinically normal areas (n=5) of scalp in medium E199 supplemented with 20% foetal bovine serum. Confluent 75cm^2 dishes of cells at passage 4 were grown in serum-free medium for 24h before a 2h incubation with 5nM [1,2,6,7 ^{3}H]-testosterone. After the addition of unlabelled carrier and ^{14}C-marker steroids, the medium and cell extracts of each cell line were analysed individually by two TLC systems and recrystallisation to a constant ^{3}H/^{14}C ratio.

Although the primary cultures were much more difficult to establish and grow than normal scalp DP cells their morphological properties were similar. After 2h incubation with testosterone no other steroids were present in significant amounts in the medium. Nevertheless, not only testosterone but also androstenedione, 5α-androstanedione and 5α-dihydrotestosterone (DHT) were identified inside the cells.

These results are rather unexpected. Our previous experiments with DP cells have found their 5α-reductase ability reflected hair growth in 5α-reductase deficiency; beard cells produced DHT but not non-balding scalp or pubic cells. Although some 5α-reductase activity was present in balding DP cells, the small amounts of DHT present may suggest it is not the active androgen in balding follicles.

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ESTABLISHMENT AND CHARACTERIZATION OF A HUMAN CELL LINE HAVING BOTH SURFACE MARKERS OF MONOCYTE-MACROPHAGE AND NATURAL KILLER CELL LINEAGE FROM A PATIENT WITH ATOPIC DERMATITIS.
Yoshiaki Hamamoto, Kou Nagai, Hiroko Furumoto, Masahiko Muto and Chidori Asagami, Department of Dermatology, Yamaguchi University School of Medicine, Ube, Japan

In general, large amounts of homogenous cells are not always available for *in vitro* studies of inflammatory skin disorder, such as atopic dermatitis. To obtain useful model systems, we tried to establish a cell line from peripheral blood of a patient with atopic dermatitis having an increased serum level of total IgE. About 2 months after cultivation of mononuclear cells, they became to proliferative in suspension. This line, termed YAA, showed a positive reaction for α-naphthyl butyrate esterase which was completely inhibited by sodium fluoride. YAA cells had surface markers reacted with CD4, CD11b and CD33 but neither with CD2, CD3 nor CD8. Interestingly, YAA cells had CD56 molecule, one of the markers specific for natural killer cells. Moreover, YAA cells produced considerable amounts of tumor necrosis factor-α when treated with phorbol ester. These findings suggest that YAA cells have some properties specific for the monocyte-macrophage lineage. This new line might be valuable for studying not only the pathogenesis of atopic dermatitis and the regulation of monocyte-macrophage differentiation but also the origin of natural killer cells.

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DIFFERENTIATION PATHWAYS OF DENDRITIC CELLS IN ACUTE VS. CHRONIC IMMUNE-MEDIATED DERMATOSES: A UNITARY CONCEPT.
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The number and distribution of skin dendritic cells, namely CD1a+ Langerhans cells (LC) and factor XIIIa+/CD36+/CD1a- dermal dendrocytes (DD), are clearly modified in most immune-mediated dermatoses. We have analyzed by immunohistochemistry and electron microscopy (EM) the lesional skin of patients with acute (erythema multiforme, acute graft-versus-host disease) and chronic immune-mediated dermatoses (lichen planus, chronic discoid lupus erythematosus, atopic dermatitis), with the aim to assess in vivo the differentiation pathways of dendritic cells in case of rapid vs. gradual influx of precursor cells into the skin. In all studied dermatoses, both LC and DD (with EM features of dendritic macrophages) were equally increased in number suggesting a possible mutually modulating role. Besides these cells, we constantly found perivascular, bluntly dendritic cells, with some flat cisternae of RER, relatively wide Golgi apparatus and few primary lysosomes. On immunohistochemistry, cells corresponding for shape and location to the above expressed CD14 antigen dimly in acute disorders, and strongly in chronic ones. No cells with intermediate EM features between LC and DD were observed. These data suggest the hypothesis that both LC and DD may differentiate in the skin from CD14 dimly +, circulating myeloid precursors. If their influx is gradual and chronic, these precursor cells are likely to enhance their expression of CD14 antigen.

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ISOLATION AND CHARACTERIZATION OF ENDOTHELIAL CELLS FROM INFANTILE HEMANGIOENDOTHELIOMA.
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The factors that induce either the growth or regression of hemangioma are not understood. To obtain information on the mechanisms that control the growth of endothelial cells in hemangioma, we have developed methods to isolate endothelial cells, maintain them in culture, and have compared the characteristics of these cells with normal dermal microvascular endothelial cells.

Four cases of infantile hemangioendothelioma (IHE) was used as culture material in this study. Endothelial cells were isolated from split thickness sections cut with a Castroviejo keratotome and then released from the sections by enzyme digestion.

Cells isolated from IHE were comprised of two distinct types of morphology, one in epithelioid and the other in spindle shape. Each of these types of cells showed a different expression of factor VIII and factor XIIIa. Hence, cells isolated from IHE were strickingly different from normal dermal microvascular endothelial cells in the respect of morphology and expression of biological markers. These unique properties of isolated endothelial cells may provide new insights into the treatment of hemangioma.

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EFFECTS OF ANTI-FAS MONOCLONAL ANTIBODY ON THE PROLIFERATION AND CYTOTOXICITY FOR CULTURED HUMAN MALIGNANT MELANOMA CELL LINES.
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Fas antigen is a cell surface protein that mediates apoptosis. Although it is expressed in various human tissues and cell lines, the detailed anti-tumor effects of anti-Fas monoclonal antibody against human malignant melanoma have not been previously described. In the present study, we investigated the effects of anti-Fas monoclonal antibody on the proliferation and cytotoxicity for six human malignant melanoma cell lines. Through flow cytometry analysis, [^{3}H]-thymidine incorporation assay and electron microscopic studies, we found that a) four of six melanoma cell lines (SK-MEL-28, G361, CaCl 74-36 and CaCl 78-1) showed positive reactivities with anti-Fas, although the percentage of anti-Fas positive cells varied from 21.2% (SK-MEL-28) to 91.2% (CaCl 78-1); b) the expression of Fas antigen on melanoma cell line G361 was slightly enhanced by IFN-γ; c) proliferation responses of Fas positive cell lines were significantly inhibited by anti-Fas in a dose dependent manner; d) Fas positive cell lines were sensitive to the cytolytic activity of anti-Fas, whereas Fas negative cell lines were insensitive; and d) electronmicroscopically, typical apoptotic changes were observed in anti-Fas treated melanoma cells. These results suggest that apoptosis-mediated Fas antigen may play an important role in tumor kinetics of malignant melanoma.

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REGRESSION IN BASAL CELL CARCINOMA. Michelle J. Hunt, Gary M. Halliday and Ross StC. Barnetson, Department of Dermatology, University of Sydney, Sydney, N.S.W., Australia

Spontaneous regression of some cutaneous tumours is well recognised, and is thought to result from an immunological response to the tumour. Regression has previously been noted in basal cell carcinomas (BCC) but no prior investigations defining the role of the immune response in the regression of this malignancy have been performed. We have examined 45 primary (BCC) (20 nodular, 25 superficial) and identified the cellular phenotypes and activation states of the cells infiltrating primary regressing and non-regressing BCC by immunocytochemistry. We have found a significantly increased numbers of CD3+ and CD4+ T cells infiltrating regressing compared to non-regressing tumours, and the expression of interleukin-2 receptor (an early activation marker for T cells) was also increased. There were no significant differences in the number of class II MHC expressing cells, Langerhans cells (CD1) or macrophages. These findings are consistent with activated CD4-positive cytokine-secreting cells being important in mediating the spontaneous regression of BCC.

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PRELIMINARY STUDY ON IMMUNOTHERAPY FOR ADULT T-CELL LEUKEMIA — INDUCTION OF HUMAN T LYMPHOTROPIC VIRUS TYPE I-SPECIFIC CYTOTOXIC T LYMPHOCYTES. Yoshihiko Katahira[1], Mitsuru Setoyama[1], Tamotsu Kanzaki[1], and Shunro Sonoda[2], Department of [1]Dermatology, [2]Virology, Faculty of Medicine, Kagoshima University, Kagoshima, Japan

Prognosis of adult T-cell leukemia (ATL) is very poor, and non of therapies have been successful to induce complete remission. Thus, more specific and stronger therapies are demanded to treat ATL. Immunotherapy, such as vaccination or infusion of cytotoxic T lymphocytes (CTLs), would be the one. We tried *in vitro* induction of CTLs from peripheral blood lymphocytes (PBLs) 1 healthy individual (HI), 4 human T lymphotropic virus type I (HTLV-I) infected asymptomatic carriers (ACs), and 3 ATL patients. PBLs were stimulated with a HLA matched HTLV-I-infected T-cell line in RPMI-1640 medium supplemented with 10% FCS, rIL-1, rIL-2, rIL-4, rIL-6 and rIL-7. HTLV-I-infected T-cell line was inactivated with heat treatment (56℃, 30min) before stimulation. These combinations were found to be the best to obtain CTL after various trials. Four weeks later, responder cells were harvested, and screened for cytotoxic activity to various HTLV-I-infected T-cell lines and B-cells expressing HTLV-I proteins. As results 5 HLA-A24 restricted $CD8^+$ CTLs and 5 HLA-DR15 restricted $CD4^+$ CTLs were established from 1/1 HI, 4/4 ACs and 1/3 ATL patient. Each CTL recognized one or two HTLV-I specific epitopes. Besides, one HLA unrestricted $CD4^+$ CTL obtained from ATL patient did not recognize HTLV-I gene products, but it killed ATL cells. These results suggested that the HTLV-I specific CTLs induced by the method described above might be useful for specific immunotherapy for ATL patients.

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IDENTIFICATION OF CENTROMERE ANTIGENS OF DIFFERENT MOLECULAR WEIGHTS BY SERA FROM ANTICENTROMERE ANTIBODIES (ACA)-POSITIVE PATIENTS: CLINICAL SIGNIFICANCE AND DISEASE SPECIFICITY. Heng-Leong Chan, Yu-Shung Lee, Hong-Shang Hong, Tseng-tong Kuo*, Departments of Dermatology and *Pathology, Chang Gung Memorial Hospital, Chang Gung Medical College, Taipei, Taiwan

ACA is generally believed to be a specific marker for CREST syndrome. Previous investigators identified several CA with sera from patients with ACA. The results were inconclusive. The clinical significance and disease specificity of CA has not been defined. Detection of clinical significance and identification of different molecular weights of CA in specific disease was performed. CA extracted from HeLa cells were electrophoresed on SDS-PAGE and subsequent immunoblotting. Sera from 67 ACA-positive patients with autoimmune diseases, non-autoimmune diseases and normal subjects were used to recognize the corresponding CA. Three major Centromere Antigens: CA-A (17kD), CA-B (80kD) and CA-C (140kD) were identified. CA-A was detected by 95.5% (64/67), CA-B 100% (67/67), and CA-C 86.6% (58/67) of the ACA-positive sera. CA recognized by sera from ACA-positive subjects lack disease specificity and can not be used in the differential diagnosis of various diseases.

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IMMUNOCOMPETENT CELLS AND ADHESIONS MOLECULE EXPRESSION IN CUTANEOUS GRANULOMATOUS CROHN´S DISEASE. E.A. Stephansson, M. Hackzel-Bradley, M.Hedblad, Department of Dermatology, Karolinska Institute, Stockholm

Metastatic Crohn´s disease is characterized by a sarcoidal granulomatous inflammation that is identical to the primary bowel lesion. So far there have been no immunohistochemical studies of the skin changes and pathogenic mechanisms are poorly understood. Direct immunofluorescence studies on lesional skin showed no accumulation of immunoglobulins or C3 in the granulomas or vessel walls. Staining with monoclonal antibodies showed great numbers of CD1a positive cells in epidermis of the lesional skin whereas in non-lesional skin there was only a few scattered cells. Dendritic CD1a positive cells were also seen in granulomas and scattered in upper dermis. There were moderate infiltrations of CD3 positive cells in dermis perivascular and in granulomas. The majority of the cells were CD4 positive, evenly distributed in the granulomas as well CD8 positive cells. CD22 positive cells were few and there was only a few $\gamma\delta$-TCR positive cells. Cells expressing CD56 were found only around sweet glands and perivascular deep in the dermis. Keratinocytes did not express HLA-DR but HLA-DR positive cells were seen in dermis. Keratinocytes expressed ICAM-1 weakly and focally in the basal cell area but endothelial cells in dermis especially in lower dermis stained strongly with ICAM-1 in a granular pattern and especially in perivascular granulomas there were numerous ICAM-1 positive cells. The same pattern was seen in specimens stained with VCAM-1. The staining of endothelial cells with E-selectin was weak. Polymerase chain reaction was used in an attempt to detect mycobacterial DNA in the granulomas. The findings support the view that cell-mediated immune responses play an important role in cutaneous granulomas in Crohn´s disease.

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INCREASED LANGERHANS CELL DENSITY INDUCED BY SUPERNATANT FROM TRANSFORMED AND NORMAL KERATINOCYTES. Gary M. Halliday, Gabrielle O'Sullivan and Ross StC. Barnetson, Department of Dermatology, University of Sydney, Sydney, N.S.W., Australia.

Langerhans cells (LC) play an essential role in the induction of cutaneous immunity. The aim of our studies is to investigate factors which regulate LC migration into the epidermis. We have shown that supernatant from the transformed keratinocyte cell line T7, when applied to the ear surface of mice, increases the density of epidermal LC. Using a more sensitive system, whereby LC are first depleted with a corticosteroid, we have demonstrated that supernatant from primary keratinocyte cultures also increases the number of LC in murine epidermis. These factors are proteins (MW > 10KDa), not prostaglandins, as their production is inhibited by cycloheximide but not indomethacin and they are sensitive to proteolysis. It is unknown whether the factors produced by T7 and normal keratinocytes are identical. While it is most likely that these factors increase LC migration into the epidermis, this remains to be determined.

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DISTRIBUTION OF APOPTOSIS-MEDIATED FAS ANTIGEN IN NORMAL SKIN AND VARIOUS TYPES OF SKIN DISEASES. Masaki Oishi, Kazuo Maeda and Makoto Takahashi, Department of Dermatology, Sapporo Medical University School of Medicine, Sapporo, Japan

Fas antigen is a cell surface protein that mediates apoptosis. In the present study, we investigated the expression of Fas antigen on normal and pathological skin tissues. Through immunohistochemical studies, we found that a) it was widely distributed in skin components such as the lower portion of keratinocytes, epidermal dendritic cells, endothelial cells, fibroblasts, apocrine glands, eccrine sweat glands, sebaceous glands, and infiltrating lymphoid cells; b) it was strongly expressed on the keratinocytes of lichenoid eruptions seen in lupus erythematosus and lichen planus, and on the spongiotic or acanthotic epidermis seen in chronic eczema, adult T cell leukemia/lymphoma (ATLL) and atopic dermatitis; c) its expression was closely correlated with lymphoid infiltrating cells; d) it was strongly expressed in lymphoid neoplastic cells, particularly in ATLL cells, and fibroblasts seen in dermatofibroma; e) it was not detected on normal melanocytes, basal cell epithelioma cells, some malignant melanomas or any junctional nevi. These results indicate that apoptosis-mediated Fas antigen may play an important role in normal skin turnover and cell differentiation, immune regulation of skin tumors, and the pathogenesis of various skin diseases.

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NICKEL/MAGNESIUM INTERACTION IN NI SENSITIVE SUBJECTS.

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In order to study the intimate chemical mechanism of interaction between nickel ions and carrier proteins to produce a complete contact allergen we have evaluated the effect of the presence Magnesium sulphate in the positive patch tests response to Nickel sulphate. Magnesium is reported to be effecting in reducing some toxic effects of nickel ions in different biological systems. 75 nickel sensitive subjects were separately patch tested to 10 μl of $NiSO_4$ 0.1 M and to 10 μl of the following solutions according to the following plan.

Group 1 (25 subjects) $NiSO_4$ 0.1 M + $MgSO_4$ 0.1 M; $NiSO_4$ 0.1 M + $MgSO_4$ 0.3 M
Group 2 (25 subjects) $NiSO_4$ 0.1 M + $MgSO_4$ 0.3 M; $NiSO_4$ 0.1 M + $MgSO_4$ 0.5 M
Group 3 (25 subjects) $NiSO_4$ 0.1 M + $MgSO_4$ 0.5 M; $NiSO_4$ 0.1 M + $MgSO_4$ 1 M

The results were evaluated at 2 days by visual scoring according to international criteria. On testing NiSO4 with $MgSO_4$ 0.1 M there were a complete suppression or significant reduction of the reactions in about 64% of the subjects. In the presence of MgSO4 0.3 M the reaction to NiSO4 was suppressed or reduced in 82% of the patients, and about 88% of the positive subjects have a suppressed or reduced reaction in the presence of MgSO4 1M. On the contrary on testing with NiSO4 plus MgSO4 0.5M about 64% of the patients had an significantly increased reaction. Our results demonstrate that MgSO4 is able to inhibit the patch tests reactions to NiSO4 in a dose dependent manner at least for 0.1, 03 and 1 M concentrations. On testing with $MgSO_4$ 0.5 M a paradoxical effect was seen. These results may be due to a competition between ions for the active sites of carrier proteins or for intracellular transport and underline the role of the chemical reactivity of the allergens in inducing contact sensitivity .

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CHANGES IN CD4+ T CELL SUBSETS (Th-1, Th-2) IN SKIN LESIONS OF PATIENTS WITH ATOPIC DERMATITIS. Takao Fujimura, Akira Fujioka, Yuko Hamada, Mikio Masuzawa, and Shigeo Nishiyama, Department of Dermatology, Kitasato University School of Medicine,Sagamihara, Kanagawa,Japan

Skin lesions of atopic dermatitis were examined for the cytokine expression using RT-PCR method. The profile of mRNA for various cytokines revealed that both Th1 and Th-2 type of $CD4^+$ T cells, probably including Th0 type, are generally infiltrating the skin lesion. In the lesion improved after contracting varicella, expression of Th1 type cytokines predominated, while Th2 type was dominantly detected in the exacerbated lesion induced by Staphylococcal infection or stimulation by mite antigens. The peripheral blood T cells from patients were shown to differentiate into Th2 type cells upon stimulation with those antigens mentioned above. (ELISPOT method). It is suggested that patients with atopic dermatitis always have highly reactive $CD4^+$Tcells infiltrating their skin, and that the switch to Th1 or Th2 dominancy is related to the outcome of the lesion, improvement or exacerbation.

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CELL SURFACE-LOCALIZED HSP72/73 PLAYS A ROLE IN ANTIGEN PRESENTATION. Gian Carlo Manara (1), Lucilla Badiali (2), Gianandrea Pasquinelli (2), Corrado Ferrari (3), Silvia Antonella Garatti (1), Giuseppe De Panfilis (4) and Paolo Sansoni (5), Depts. of (1) Dermatology, (3) Pathology, and (5) Medicine, University of Parma, Parma; (2) Institute for Clinical Electron Microscopy, University of Bologna, Bologna; (4) Division of Dermatology, Brescia Hospital, Brescia; Italy

The peptide binding protein PBP72/74, serologically related to the human constitutive HSP73 kD, has been recently proposed to play a role in antigen presentation in mice. The present study was intended to verify whether human antigen presenting cells express the HSP73 kD and to assess its role in antigen processing.

Purified monocytes and B cells lysates were subjected to western blot analyses. Moreover, both cell types were processed for TEM and SEM immunoelectron microscopy (IEM). Finally, the potential of an anti-HSP72/73 MoAb to affect antigen presenting-capability was assessed by using viable as well as fixed monocytes and B cells.

Western blot analyses showed that both human monocytes and B cells constitutively possess the HSP72/73. IEM studies provided evidence for the cell surface localization of HSP72/73. Finally, functional studies demonstrated that anti-HSP72/73 MoAb impaired the capability of viable cells to present a recall antigen and of fixed cells to present a synthetic peptyde.

The present study demonstrates that human monocytes and B cells possess and express on their cell surface a member of the HSP70 family. Moreover, functional data provide evidence for a critical role of the surface localized HSP70 molecules in antigen presenting.

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CUTANEOUS STROMAL CELLS SUPPORT PROLIFERATION OF THY-1+ DENDRITIC EPIDERMAL T CELLS (Thy-1+ DEC). S. Nakagawa, S. Aiba, H. Ozawa, H.Tagami, Department of Dermatology, Tohoku University School of Medicine, Sendai, Japan.

It is well known that the murine epidermis contains Thy-$1^+$$CD4^-$$CD8^-$$\gamma/\delta TcR^+$ T cells referred to as Thy-1^+ DEC. They are exclusively $V\gamma5TcR^+$ and can be activated in vitro only with the combination of Con A and IL-2. Recently we have reported the presence of other $\gamma\delta TcR^+$ T cell subsets (i.e. $V\gamma5TcR^-$) in the skin which migrate with Langerhans cells during the skin organ culture into the culture media. The latter can be expanded in the coculture with the established fibroblast-like cutaneous stromal cell (CSC) clone 12E2 and IL-2 (Nakagawa et. al. Eur J Immunol in press). The purpose of this study was to elucidate that 12E2 can also support the proliferation of Thy-1^+ DEC. Epidermal cell suspensions were positively selected by anti-Ly5 antibody and anti-mouse immunoglobulin-coupled magnetic beads. These $Ly5^+$ cells (5×10^4 /well) were plated onto12E2 monolayer which were seeded (2×10^4/well) 2 days before and cultured in the presence or absence of 100 U/ml IL-2. A marked proliferation of small lymphoid cells was observed in the coculture of 12E2 with IL-2. Neither 12E2 alone nor IL-2 alone could induce a significant growth of the cells. Flow cytometry of the proliferating cells 14 days after coculture revealed that they were Thy-$1^+$$CD3^+$$CD4^-$$CD8^-$$\alpha\beta TcR^-$$\gamma/\delta TcR^+$ $V\gamma5TcR^+$. These data indicate the unique capacity of the CSCs to support the growth of Thy-1^+ DEC.

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CLINICAL AND IMMUNOHISTOLOGICAL INVESTIGATIONS ON SKIN LESIONS OF ACUTE GRAFT-VERSUS-HOST DISEASE AFTER BONE MARROW TRANSPLANTATION. Kaoru Ito, Junko Izumi and Masaaki Ito, Department of Dermatology (KI, JI, MI) and Niigata University Bone Marrow Transplantation Team, Niigata University School of Medicine, Niigata, Japan

Mechanism in the development of skin lesions in acute graft-versus-host disease (GVHD) has not been completely understood. In the present study, we evaluated clinically and immunohistologically the skin lesions of 38 cases who developed acute GVHD among 69 patients with bone marrow transplantation during 1983 and 1992. Skin lesions from 23 patients were investigated immunohistologically using monoclonal antibodies against CD1a, CD2, CD3, CD4, CD5, CD8, CD11a, CD11b, CD16, CD54 (ICAM-1), CD56, CD57, $\gamma\delta$TCR and HLA-DR.

Most patients developed skin lesions from 6 to 15 days after transplantation. Eruptions started most frequently from their palms and soles. Pathological grading of skin lesions showed grade II in most of the cases.

CD4/CD8 ratio of infiltrating mononuclear cells in epidermis and dermis varied among cases. However, CD4+ cells tended to predominate over CD8+ cells in the early stage and CD8+ cells increased in the late period. With natural killer (NK) cell markers, CD16+ cells were observed in 16 cases. In every case, HLA-DR was expressed on keratinocytes and epidermal CD1a+ cells were reduced. Keratinocytes in 22 cases showed ICAM-1.

Substantial infiltration of CD4+ cells in the epidermis and dermis suggest the participation of other mechanism than major or minor histocompatibility antigen mismatch. Expression of HLA-DR antigen, ICAM-1 and the presence of NK cells may play some roles in the condition. Increase of CD8+ cells in the late stage could be induced by CD4+ cell or autoreactive T cells which were produced by the failure of tolerance.

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CUTANEOUS EXPRESSION OF THE B7 ACTIVATION AG AND ITS LIGAND CD28: RELEVANCE TO IMMUNE RESPONSES GENERATED IN SKIN. J.C.Simon[1], A.Dietrich[1], V.Mielke[2], M.Augustin[1], W.Vanscheidt[1], J.A.Ledbetter[3], P.S.Linsley[3], E.Schöpf[1] and W.Sterry[2], Depts. of Dermatology, Freiburg[1], Ulm[2], Germany and [3]Bristol-Myers Squibb, Seattle, WA, U.S.A.

Interactions of CD28 on T cells with its ligand B7 on APC have been shown to activate T cells via an MHC/Ag-independent "alternative" pathway. The in vivo relevance of these molecules for cutaneous immunity is presently unknown. This prompted us to study the expression of B7 and CD28 in allergic contact dermatitis (ACD n=5), lichen planus (LP n=5) mycosis fungoides (MF n=3), pleomorphic T cell lymphoma (PTCL n=4), cutaneous B-cell-lymphomas (BCL n=4) and in normal human skin (NS n=4). Serial cryostat sections were stained with a panel of mAb directed against CD28 and B7 using immunohistochemistry (ABC-technique). CD28 was expressed by the majority of dermal and epidermal T cells in ACD, LP, MF, PCL and on accompanying T cells in BCL. In ACD, LP, MF and PTCL, B7 expression was found on dermal dendritic cells, on dermal macrophages, on epidermal Langerhans cells and on some keratinocytes (KC). Close contact of $CD28^+$ T cells and $B7^+$ epidermal cells was observed in areas with histological signs of cytotoxic damage. In BCL, B7 was also expressed by the tumor cells. In NS only few perivascular T cells expressed CD28, no B7-staining could be detected. We conclude, that CD28 is expressed by the majority of T cells infiltrating the skin of patients with ACD and LP, and that cutaneous T cell lymphomas uniformly express CD28. B7 staining was observed predominantly on professional cutaneous APCs and on some KC. Based on these findings, we speculate that B7/CD28 signalling may contribute to T cell-activation and CTL induction in skin.

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PROLIFERATIVE RESPONSES OF PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) TO CANDIDA ALBICANS ANTIGEN, PHA AND SUPERANTIGENS IN ATOPIC DERMATITIS PATIENTS--ANERGY IN DELAYED TYPE HYPERSENSITIVITY TO C. ALBICANS ANTIGEN. Misako Tanaka, Setsuya Aiba, Hachiro Tagami, Department of Dermatology, Tohoku University School of Medicine, Sendai.

Elliott and Hanifin previously reported that atopic dermatitis (AD) patients showed normal lymphocyte transformation responses to C. albicans and streptokinase-streptodornase antigens, whereas their cutaneous responses to those antigens were significantly diminished. We have also reported that AD patients manifested decreased cutaneous response to C. albicans antigen by patch tests. The purpose of this study was to confirm that AD patients showed normal lymphocyte transformation responses to C. albicans antigen. PBMC from 21 AD patients and 14 HC subjects were cultured with optimal concentrations of C. albicans antigen, and PHA or superantigens for 5 and 3 days, respectively, and [^{3} H]-thymidine incorporation for the final 16 hrs of culture was assessed. Unexpectedly, AD showed a significantly lower response to C. albicans antigen (22,129 $\pm$ 28,816) than HC subjects (59,997 $\pm$ 40,311, $p < 0.01$), although there was no statistically significant difference in responses to PHA or superantigens between AD patients and HC subjects. This decreased response was reversely correlated with RAST scores for C. albicans antigen in AD patients. These data clearly demonstrated the presence of in vitro anergy to C. albicans antigen in AD patients.

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GAMMA/DELTA T CELL RECEPTOR-POSITIVE CELLS OF HUMAN SKIN: APPEARANCE IN DELAYED-TYPE HYPERSENSITIVITY REACTION. Mayumi Fujita[1,2], Yoshiki Miyachi[3] and Sadao Imamura[1], [1]Department of Dermatology, Kyoto University Faculty of Medicine, Kyoto, Japan, [2]Department of Dermatology, University of Colorado School of Medicine, Denver, U.S.A. [3]Department of Dermatology, Gunma University School of Medicine, Gunma, Japan

In order to investigate the distribution and involvement of human gamma/delta T cell receptor (TCR)$^+$ cells in delayed-type hypersensitivity reaction of the skin, we have examined the occurrence and kinetics of gamma/delta TCR$^+$ cells during skin reactions of allergic contact dermatitis. In allergic contact dermatitis to DNCB, increased gamma/delta TCR$^+$ cells were observed both in the epidermis and the dermis from 48 hours after the challenge and most of them were Vδ1$^-$ Vδ 2$^+$ V 9$^+$. The percentage of gamma/delta TCR$^+$ cells in the peripheral blood remained unchanged and a few gamma/delta TCR$^+$ cells in the skin lesions proliferated in situ. It is suggested that the gamma/delta TCR$^+$ cell in skin lesions of allergic contact dermatitis may not be involved in initiation of delayed-type hypersensitivity but may have some other roles responding to factors induced in the reactions.

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GRAFT-VERSUS-HOST DISEASE IN NUDE MICE. Hideki Morita, Takako Kihara, Mika Hori and Yukio Kitano, Department of Dermatology, Hyogo College of Medicine, Hyogo, Japan

Graft-versus-host disease(GVHD) is induced by a complex series of immunologic events following transfer of immunocompetent T cells into an immunoincompetent host. In this study, we examined what factors influenced the development of GVHD in nude mice.

BALB/c nude mice injected with MHC-disparate B6 spleen cells exhibited only transient GVHD. Transient splenomegaly occurred and returned to normal 2 weeks after transfer. No animals died during the period of observation. Inoculation of B6 spleen cells into BALB/c nude mice produced alloantibodies to the donor cells. These allo-antibodies eliminated host-MHC-reactive donor T cells from the host. Prevention of GVHD was achieved by the pretreatment of spleen cells with anti-Thy-1 antibody or anti-CD4 antibody and complement. This was not done by anti-CD8 antibody and complement. Thus the nude mouse is considered to be a useful recipient for clarifying the mechanisms involved in GVHD.

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INDUCTION OF CELLULAR IMMUNITY TO TL ANTIGEN BY SKIN GRAFT AND ITS EFFECT ON *IN VIVO* GROWTH OF TL$^+$ LYMPHOMA. Akimichi Morita[1], Toshitada Takahashi[2], Takuo Tsuji[1] and Yuichi Obata[2], [1]Department of Dermatology, Nagoya City University, School of Medicine and [2]Laboratory of Immunology, Aichi Cancer Center Research Institute, Nagoya, Japan

Mouse TL antigens belong to the family of MHC class I antigens. However, the expression of TL in normal mice is restricted to thymus and intestinal epithelium. Certain mouse strains, such as B6 and C3H, do not express TL in normal thymus, but T-cell lymphomas arising in these TL$^-$ mice often express TL. While classical class I antigens function as antigen-presenting molecules to the T-cell receptor (TCR), the function of TL is yet to be defined. Furthermore, TL antigens have not been known to be targets of cytotoxic T cells or to mediate graft rejection.

To elucidate the function of TL, we have derived transgenic mice expressing TL in most tissues including skin by introducing TL gene of B6 origin driven by the H-2K^b promoter. By grafting their skins on C3H and (B6 X C3H)F$_1$ mice and testing cellular immune response to TL, the following conclusions were obtained: (1) TL antigens on skin behave as a transplantation antigen. (2) TL antigens elicit a TCR$\alpha\beta^+$ CD8$^+$ cytotoxic T cell (CTL) response. (3) TCR recognize TL directly without antigen presentation by H-2 molecules. (4) The CTL lyse TL$^+$ lymphomas of various H-2 haplotype. (5) (B6 X C3H)F$_1$ mice recognize TL antigens on B6 TL$^+$ leukemia.

To examine whether cellular immunity to TL has any effect on the growth of TL$^+$ lymphoma *in vivo*, TL$^+$ leukemia cells were inoculated into the F$_1$ mice in which the immunity to TL was previously induced by grafting TL$^+$ skin from the transgenic mice. The F$_1$ mice showed significant resistance to the tumor growth and prolonged survival. The immunity to TL antigen thus induced demonstrated the anti-tumor effect *in vivo*.

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INHIBITION OF INTERLEUKIN-8-INDUCED HLA-DR EXPRESSION ON HUMAN KERATINOCYTES BY ANTIPSORIATIC DRUGS. Lajos Kemény*, Edit Olasz*, Anna Sz. Kenderessy*, Günter Michel+, Axel Beetz+, Thomas Ruzicka+, Attila Dobozy*, Department of Dermatology University of Szeged, Hungary* Department of Dermatology University of Munich, FRG+

Our recent results suggest that interleukin-8 (IL-8) may directly influence several functions such as chemotaxis and HLA-DR expression of human keratinocytes. The aim of the present work was to study whether the IL-8 induced HLA-DR expression of keratinocytes might be modulated by antipsoriatic drugs. Freshly separated and cultured normal human keratinocytes were used for the investigations. Cells were stimulated by 10 nM IL-8 for 24 or 48 hrs at 37 °C and the HLA-DR expression on keratinocytes was determined by flow cytometry. According to our results IL-8 significantly induced the expression of HLA-DR antigen both on freshly separated and cultured human keratinocytes. This IL-8-induced expression of HLA-DR molecule could be significantly inhibited by antipsoriatic drugs such as cyclosporin, FK 506, dithranol, calcitriol and calcipotriol. These results indicate that IL-8-induced HLA-DR antigen expression on the cell surface of human keratinocytes may be of significance in the pathogenesis of inflammatory skin diseases such as psoriasis. Furthermore, our studies might be of benefit in a more detailed understanding of the mechanism of action of antipsoriatic drugs.

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DELAYED-TYPE HYPERSENSITIVITY(DTH)-INITIATING CELLS IN CUTANEOUS NICKEL SULFATE HYPERSENSITIVITY. Norihisa Ishii[1], Yasuyuki Sugita[1], Hiroshi Nakajima[1] and Philip W. Askenase[2], [1]Department of Dermatology, Yokohama City University School of Medicine, Yokohama, Japan, and [2]Department of Internal Medicine, Yale University School of Medicine, New Haven, CT.

We show that early-acting(2 hr) DTH-initiating cells were present and required for the expression of the later-acting, 24 hr DTH effector T cells that mediate skin swelling responses in the $NiSO_4$ DTH system. DTH-initiating cells were required for elicitation of subsequent 24 hr $NiSO_4$ DTH, and had a phenotype for an antigen-specific cell(Thy1$^+$, CD3$^-$4$^-$5$^+$8$^-$23$^+$, B220$^+$, IL-2R$^-$, IL-3R$^+$). The DTH-initiating cells were found to be necessary for local recruitment of DTH-effector cells. Relative high doses of anti-B220(CD45RA) and anti-CD23(IgE FcRII) MoAb were necessary to completely eliminate all DTH-initiating cells, and therefore completely block subsequent expression of late 24 hr DTH activity. In addition, we found that mast cells are important for expression of DTH-initiating cell activity in the murine $NiSO_4$ DTH system. Defective DTH-initiation in mast cell deficient WBB6F$_1$-W/W^v is probably due to the microenvironmental absence of mast cells, rather than a failure in generation of DTH-initiating cells. Our results indicate that two different antigen specific Thy-1$^+$ cells are necessary to elicit $NiSO_4$ DTH in mice, and that mast cells are necessary for expression of the early component that is due to the DTH-initiating cell activity.

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EOSINOPHIL-MEDIATED CYTOTOXICITY: MODULATION BY CYTOKINES. TP Birkland, D Solecki, AR Oseroff, and SH Pincus. Department of Dermatology, SUNY at Buffalo and Roswell Park Cancer Institute, Buffalo, NY, USA.

Eosinophils (Eos), bone-marrow derived cells often found at mucosal surfaces and in skin, may actively participate in resistance to tumors. We have previously established that Eos can be tumoricidal. We tested the hypothesis that Eos-mediated cytotoxicity can be modulated by cytokines. Peripheral blood Eos from normal volunteers and one hypereosinophilic syndrome patient (HES) were purified by negative selection using anti-CD16 magnetic beads and added to overnight cultures of adherent FaDu (human squamous cell carcinoma) cells. Cytokines (log_{10} dilutions of IL-2, IL-3, and γ-IFN) were added concurrently with Eos (10:1, Eos:FaDu). Cultures were pulsed with ^{3}H-thymidine (^{3}H-T) after 48 hr. Cytotoxicity was defined as the difference between ^{3}H-T uptake of FaDu cultures with and without Eos and ± cytokines.

	% decrease in ^{3}H-T uptake			(* denotes $p<0.05$)
Cytokine	0	10	100	1000 pM
γ-IFN (HES Eos)	2.0±1.7	13.1±3.4*	9.2±2.3*	20.8±3.0*
γ-IFN	9.0±2.6	13.5±2.9	9.8±3.8	10.0±1.9
IL-3	11.1±4.6	2.3±5.1*	1.0±5.1*	-2.3±3.9*
IL-2	11.0±1.8	11.5±1.5	13.1±2.6	4.1±3.3

Cytotoxicity of HES Eos was significantly enhanced by γ-IFN ($p<0.05$), while normal Eos were not affected. IL-3 significantly reduced cytotoxicity by normal Eos, while IL-2 had no effect. Our studies establish that cytokines can modulate Eos-mediated cytotoxicity. These results suggest that locally produced cytokines may act to enhance tissue tumoricidal actions of Eos.

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KERATINOCYTES IN PSORIATIC PLAQUE OVER-EXPRESS THE RECEPTOR FOR NERVE GROWTH FACTOR. N Tidman, L Churchill, I M Leigh and I McKay. Department of Experimental Dermatology, London Hospital Medical College, London, UK

Normal human keratinocytes, *in vitro*, produce nerve growth factor (NGF) and express its receptor (NGF-R). NGF acts as a mitogen for them suggesting it stimulates keratinocyte proliferation through an autocrine pathway. The aim of this study was to determine whether such an autocrine loop may play a role in regulating keratinocyte proliferation in psoriatic plaque keratinocytes *in vivo*. To this end we have first examined the distribution of NGF-R in normal skin and in psoriatic plaque, using three stage immunofluorescence staining, with two different monoclonal antibodies showing specificity for the 75 kD NGF-R. Like others we have determined that expression of the receptor is patchy in normal epidermis and depends on the body location. In hairy and non-hairy skin the expression is generally weak and restricted to basal cells, giving plasma membrane like staining. The staining is more obvious in the roof of dermal papillae in hairy skin and outer epithelial root sheath of hair.. In psoriatic plaque the staining intensity is increased and basal cells are uniformly positive all the way down to the base of the rete pegs. We conclude that the distribution of NGF-R is altered and the level of receptor expression is elevated in psoriatic plaque keratinocytes. We therefore suggest that NGF may be involved in the autocrine stimulation of keratinocyte growth in psoriatic plaque.

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EICOSAPENTAENOIC ACID SUPPRESSES SYNTHESES OF CYTOKINES IN MONONUCLEAR LEUKOCYTE AND INTERLEUKIN-8 IN LESIONAL SKIN OF PSORIASIS. Atsushi Mochida, Takashi Terano*, Kei Kuroda, Masaru Fujita, Shoji Okamoto and Takayuki Kojima. Department of Dermatology and Second Department of Internal Medicine*, School of Medicine, Chiba University, Chiba, Japan

Eicosapentaenoic acid (EPA) has been reported to have a beneficial effect on psoriasis (PSO). Recent works have revealed the role of cytokines including interleukin-8 (IL-8) in the inflammatory process of this disease. It will provide a clue to understand the pathogenesis of psoriasis to clarify the effect of EPA on cytokine production. In this study EPA ethylester at a daily dose of 3.6g was administrated to nine psoriatic patients for 12 weeks without changing their topical treatment. Every 4 weeks we evaluated their clinical states with psoriatic area and severity index (PASI) score, and measured IL-1α , IL-1β , IL-6 and IL-8 formation in mononuclear leukocyte (MNL) stimulated by Con-A. Before and after administration of EPA, biopsies were taken from lesional skin to detect IL-8 by a polyclonal anti-IL-8 antibody. The mean PASI score decreased from 13.7 to 4.9 during EPA treatment. Syntheses of IL-1α , IL-6 and IL-8 in MNL from psoriasis were elevated above normal levels and reduced significantly after EPA treatment as indicated below.

	IL-1α	IL-6	IL-8	
Healthy subjects	1.69	19.7	61.1	IL-1α , IL-β : fmol/10^6 cells
PSO before treatmet	2.45	50.7	233.1	IL-8 : ng/ml
PSO after treatment	1.48	24.6	92.6	

IL-8 was detected immunohistochemically in the corneal layer especially in microabscess and slightly in the basal layer before treatment and was almost undetectable after EPA treatment. These results suggest that the suppression of cytokine synthesis of MNL and keratinocyte is one of the mechanisms by which EPA effects on psoriasis.

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THE CONCEPTION OF CYTOKIN-ADHESIN PATHOGENESIS OF PSORIASIS AND ITS FURTHER PERFECTION. Andris Rubins, Hackel Weksler, Ilona Hartmane, Yuris Lielbriedis, Chair of Dermatovenerology, Latvian Academy of Medicine, Riga, Latvia

An attempt has been made to perfect the conception of cytokin-adhesin pathogenesis of psoriasis advanced by the authors generalizing the results obtained from further research into various forms of the disease (vulgaris, erytrodermatic, arthropatic) in 112 patients.

Monoclonal antibodies (MA) have been used to T-lymphocyte integrin-adhesin proteins, immunocyte $CD3^+$, $CD4^+$, $CD8^+$, $CDlla^+$. Also LFA-1 (LAI test) and modulation peculiarities of immunocyte reception concerning interferon γ (IFN-γ), interleukin 2 (IL-2) and tumor necrotic factor TNF-α (LAI modulation test) as well as intracutaneous test with FHA and mycobacteria.

It has been proved that particularly in erytrodermatic and arthropatic psoriasis the activity of illness correlate with adhesin $CD3^+$, $CDlla^+$, $CD4^+$, $CD8^+$, as well as reaction increase in blood T-lymphocyte cytokins — LFA-1, IFN-γ and TNF-α. Sensibilization of psoriatic dermal T-lymphocytes to mycobacteria has been determined.

Based on these data one may assume that cytokins, IFN-γ and TNF-α play the decisive role in the trigger mechanism of psoriasis pathogenesis inducing the formation of corresponding cytokin and adhesin receptors of T-lymphocytes. Taking into consideration the fact that we and other authors have detected sensibilization of psoriatic dermal T-lymphocytes to mycobacteria, it is possible that one of the inductors of psoriasis autoimmunity is heat shock (stress) protein possessing properties of mimicry antigen cross-like to mycobacterial and keratonocyte antigens of the patients' tissues.

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IMMUNOHISTOLOGICAL DEMONSTRATION OF INTERLEUKIN-2 IN PSORIATIC SkIN LEISIONS. Liu Wei, Cai Rui-kang and Zhao Qing-li, Department of Dermatology, General Hospital of Air Force, Bejing, 100036, China

Interleukin-2, an important mediator of immune system, was studied on frozen sections from psoriatic skin by immunohistochemical technique. Using murine monoclonal antibodies against human interleukin-2, the cytokine was demonstrated in epidermis, apparently in cytoplasma of keratinocytes. Large amount of epidermal cells were stained, especially in the proliferating region of stromal cell. Mononeuclear cells in papillary dermis were also positive partly. Quantitative studies by microspectrophotometer show that, epidermal cells in active psoriatic plaque from 15 patients stained heavily for interleukin 2 as comparied with stationary psoriatic skin from other 15 patients, normal human skin from 15 healthy controls gave a nagetive staining in the same experiment. Satistatical difference exist between the all three groups. These results indicated an expression of interleukin-2 in psoriatic skin leisions, and correlated with clinical and pathologic courses of the disease.

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IMMUNOHISTOCHEMICAL LOCALIZATION OF γ-INTERFERON IN SKIN LEISIONS OF PSORIASIS. Zhao Qing-li, Guan Peng-ju and Liu Wei, Department of Dermatology, General Hospital of Air Force, Bejing, 100036, China

Using murine monoclonal antibody against human γ-interferon(IFN-γ), An immunohistochemical technique (APAAP method) , was used to demonstrate the distribution of IFN—γ in cryostat sections from psoriatic skin lesions. In all 30 specimens IFN- γ was observed in keratinocytes in stratum spinosum and in stratum corneum, in some mononuclear cells and endothelial cells in the papillary dermis. The staining was in granular particle localized either in intercellular space of the epidermis or in cytoplasm of mononuclear cell and endothelial cells. The IFN-γ in the epidermis was more pronounced in sections from highly active psoriasis than that from stationary psoriatic skin. The monoclonal antibody did not stain sections from 15 healthy individuals. The findings indicated that IFN-γ was expressed in the psoriatic skin and may be of significance in the pathophysiology of psoriasis.

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QUANTITATIVE ANALYSIS OF IL-8 (INTERLEUKIN 8) mRNA EXPRESSION IN PSORIASIS BY REVERSED TRANSCRIPTASE-POLYMERASE CHAIN REACTION (RT-PCR). Hidekazu Yamada, Ryo Izutani*, Tatuo Yudate, Tomoaki Orita, Tadashi Tezuka, Department of Dermatology, *Department of Surgery, Kinki University School of Medicine, Osaka, Japan.

In psoriatic involved skin, neutrophiles are infiltrated in the epidermis. The mechanism of this phenomenon is explained by the fact that keratinocytes produce and secrete the chemotactic factor and express their adhesion molecules on the cell membranes. The aim of this study was to determine the presence of IL-8, IL-6 and IL-1α, mRNA in involved skin from psoriasis patients using the qualitative PCR, and to develop and use a quantitative PCR to determine the amount of IL-8 mRNA in involved skin. To measure the amount of IL-8 in the involved and un-involved skins of psoriasis quantitatively, IL-8 mRNA was measured using a competitive PCR method with a synthetic IL-8 RNA as an external standard that differs in size from native RNA. The percentage of psoriasis specimens expressing IL-8, IL-6 and IL-1α mRNA was increased in the involved skin in comparison with non-lesional skin of psoriasis patients under normal control. In addition, using the quantitative PCR method, there were significantly higher levels of IL-8 mRNA in the involved skin, in comparison with the one in the non-involved skins. These data suggest that IL-8 may have an important role in the augmentation of the inflammatory process in psoriatic lesional skin.

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DIFFERENT PATTERNS OF PROTEIN BINDING TO THE IL-4 CLE0 ELEMENT IN HUMAN Th1 AND Th2 CELLS. Eddy A. Wierenga[1], Monika Walchner, Elisabeth H. Weiss[2], Peter Kind, Gerd Plewig, Martien L. Kapsenberg[1] and Gerald Messer, Department of Dermatology, Ludwig-Maximilians-University of Munich; [1]Laboratory of Histology and Cellbiology, Academish Medish Centrum, University of Amsterdam, The Netherlands; [2]Institute for Anthropology and Human Genetics, L-M-University of Munich, W-8000 München 2, F.R.G.

Allergen-specific CD4+ T lymphocytes from atopic patients are mainly Th2 cells that secrete high amounts of Il-4 and Il-5, whereas allergen-specific T cells from non-atopic control individuals are Th1 cells that do not secrete these cytokines or only in limited amounts. In atopic disease, IL-4 and Il-5 play a crucial role in the induction of IgE synthesis and eosinophilia. To investigate the intracellular pathways of differential cytokine gene activation in human Th1 and Th2 cells at the level of DNA binding proteins regulating gene transcription we analyzed nuclear protein fractions isolated from well-characterized Th1 and Th2 clones 2 h after mitogenic stimulation with a combination of anti-CD3 and anti-CD28 mAb, yielding high levels of secreted cytokines.

Binding of transcription factors to radiolabeled double stranded DNA oligonucleotides containing motifs of known *cis*-activating promoter elements, found to be functional in the activation of several cytokine-encoding genes (including IL-2, IL-4, IL-5 and TNF-ß), was analyzed by electrophoretic mobility shift assays (EMSA).

Identical binding patterns of proteins from Th1 and Th2 cells were found using the NF-*k*B binding motif of the HLA-A2 gene, AP-1 binding motif of the collagenase gene and the conserved lymphokine element 0 (CLE0) and the CK-1 element (CLE1) of the IL-5 gene. An interesting difference, however, was found in the binding activity to the CLE0 element of the IL-4 promoter. In Th1 cells, the binding activity of an as yet unidentified protein was strongly upregulated upon T cell activation and correlated with the absence of substantial IL-4 mRNA after 16 h and Il-4 protein after 24 h. The same binding activity was detected in unstimulated Th2 cells and was reduced upon T cell activation, which resulted in strong signals for IL-4 mRNA after 16 h and IL-4 protein after 24 h. Our findings present the first evidence for the existence of a suppressive transcription factor, negatively regulating IL-4 transcription in human Th1 and Th2 cells.

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HUMAN RECOMBINANT IL-2 INHIBITS THE GROWTH OF *STAPHYLOCOCCUS AUREUS IN VITRO*. Dietrich Abeck, Karsten Neuber, Gerd Steuernagel and Johannes Ring, Department of Dermatology, University Hospital Eppendorf, University of Hamburg, Germany

Cytokines have been shown to exert a multitude of both pro- and antiinflammatory effects in different in vitro or in vivo conditions. Little is known about the interactions of cytokines with microbial growth. It was the purpose of this study to investigate the influence of IL-2 on the growth of *Staphylococcus aureus in vitro*. Different strains of *S.aureus* (n=5) were incubated with 10, 100 and 1000 U recombinant human IL-2 in 200 µl RPMI1640 medium overnight. The growth of bacteria was measured as number of colony forming units (CFU). The binding of IL-2 to *S.aureus* was determined by FACS analysis using phycoerythrin (PE) conjugated IL-2 and unconjugated IL-2 as simultaneous control. The incubation with IL-2 inhibited the growth of all *S.aureus* strains in a dose-dependent manner significantly ($p<0.0001$). Incubation of *S.aureus* cultured overnight with PE conjugated IL-2 at 37°C for 1 h resulted in a marked increase of fluorescence intensity. Coincubation with unconjugated IL-2 led to a specific inhibition of fluorescence intensity. The present data suggest binding of IL-2 to staphylococcal cell surface and inhibitory effects upon *S.aureus* growth. This might reveal a new mechanism of immune defense against coagulase-positive staphylococci playing a role at local sites of bacterial infection e.g. in the skin.

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PRODUCTION OF INTERLEUKIN-1(IL-1)α AND IL-8 BY CULTURED HUMAN KERATINOCYTES: REGULATION BY 1,25-DIHYDROXYVITAMIN D3(1,25(OH)2D3) AND ITS ANALOGUE MC903. Jian-Zhong Zhang•, Kohji Maruyama, Ichiro Ono and Fumio Kaneko, Department of Dermatology Fukushima Medical College, Fukushima, Japan

There has been some evidence that in psoriasis cytokine production was dysregulated, which was thought to play roles in the formation of psoriatic lesions. IL-1α is decreased and IL-8 is increased in psoriatic epidermis. Recently, vitamin D3 compounds were reported to be effective in treatment of psoriasis. The present study focuses on effects of vitamin D3 on IL-1α and IL-8 production from cultured human keratinocytes.

Our results showed that 1,25(OH)2D3 and MC903 inhibited IL-8 production from normal human keratinocytes (NHKs) induced by tumour necrosis factor(TNF)α and PMA/LPS while they showed different regulatory effects on IL-1α production. The TNFα-induced IL-1α was inhibited whereas PMA/LPS-suppressed IL-1α was recovered by 1,25(OH)2D3 and MC903. In contrast, hydrocortisone and cyclosporin A only inhibited IL-8 production, without significant effects on IL-1α production from NHKs stimulated by PMA/LPS.

These results suggested that 1,25(OH)2D3 and MC903 have biregulatory effects on IL-1α and IL-8 productions and these seem to contribute to their beneficial efficacy on treatment of psoriasis.

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A METHOD FOR THE QUANTIFICATION OF INTERLEUKIN-4 IN SERUM AND INTERLEUKIN-4 LEVELS IN PATIENTS WITH ATOPIC DERMATITIS. Masanori Kasamatsu and Takuo Tsuji, Department of Dermatology, Nagoya City University Medical School, Nagoya, Japan

Many humoral and cellular immunological abnormalities have been reported in Atopic Dermatitis (AD) . Since interleukin-4 (IL-4) enhances the IgE production and the IgE-Fc receptor expression by B cells, we hypothesized that IL-4 may play an important role in the regulation of the immune response in AD.

To evaluate the relationship between AD and IL-4, we have developed a sandwich enzyme-linked immunosorbent assay (ELISA) using chemiluminescent enzyme substrate 3-(2'-spiroadamantane)-4-methoxy-4-(3"-phosphoryloxy)phenyl-1,2-dioxetane (AMPPD) for the quantification of IL-4 in serum. IL-4 could be measured in concentrations from 0.17 pg/ml.

The IL-4 levels in 49 patients with AD (1.50 ± 1.36 pg/ml) were significantly higher than those in 69 normal controls (0.788 ± 0.624 pg/ml).

It is suggested that some humoral and celular immunological abnormalities in AD may be related to an increase in serum IL-4 levels.

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CYTOKINES AND CHEMOATTRACTANTS BY LYMPHOID CELLS STIMULATED WITH STREPTOCOCCAL ANTIGEN IN PATIENTS WITH BEHCET'S DISEASE. Fumio Kaneko, Yasuyuki Kikkawa, Kohji Maruyama and Jan-Zhong Zhang, Department of Dermatology, Fukushima Medical College, Fukushima, Japan

The etiology of Behcet's disease (BD) is still obscure, although the patients tend to have hypersensitivity against streptococcal-related antigens and some immunological abnormalities, such as dysfunction of suppressor T cells and natural killar cells in their acute phase. A biopsy from BD lesions reveals that mononuclear lymphoid cells (LCs) and polymorphonuclear neutrophils (PMNs) are infiltrated around vessels.

In this study, we have attempted to stimulate peripheral blood LCs (PLCs) from BD patients with streptococcal antigen, cell wall of S.salivarius (CWSS), to find production of cytokines and chemoattractants. PLCs were prepared from the patients and normal healthy controls and PMNs was taken from a normal healthy volunteer for chemotaxis. Cytokines were measured using ELISA and chemotaxis was assayed using a micro-Boyden chamber. The results revealed that IL-1α, IL-6 and IL-8 from PLCs stimulated by CWSS increased in a dose-and time-dependent manner and were significantly higher in BD patients than controls. The culture supernatants of PLCs stimulated by CWSS showed significant chemotactic activity in BD patients.

These data indicate that streptococci, which are normaly present in the oral cavity, cause LC stimulation and lead chemotaxis from LCs in BD patients. The organisms might act as so-called "superantigen" in BD.

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LEVELS OF INTERLEUKIN-6 IN PATIENTS WITH BEHÇET'S DISEASE. Yuko Yamakawa, Yasuyuki Sugita, Sanami Takahashi, Yasuhide Takahashi, Tetsuo Nagatani, Hiroshi Nakajima, Department of Dermatology, Yokohama City University School of Medicine, Yokohama, Japan

Behçet's disease is a chronic systemic inflammatory disease characterized by skin eruption, recurrent ulceration of the oral cavity and genitalia, uveitis and other manifestations. Although the etiology of the disease is unknown, some investigators have reported the involvement of several cytokines such as IFN-γ, IL-2, IL-6 and TNF-α in Behçet's disease.

IL-6 is an important mediator promoting inflammation. Thus we have investigated levels of IL-6 in plasma and supernatants of cultured peripheral blood mononuclear cells (PBMC) from patients with Behçet's disease by using sensitive enzyme-linked immunosorbent assay. And IL-6 gene expression was also examined in cultured PBMC by using Northern blot analysis.

Our results revealed the up-regulation of IL-6 production in active patients with Behçet's disease.

These findings imply that IL-6 may play a role in the pathogenesis of the Behçet's disease.

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SERUM CYTOKINES AND IMMUNE COMPLEXES IN PATIENTS WITH ALLERGIC CUTANEOUS VASCULITIS. Tangde Zhang, Yingquan Liang and Xanwen Qiu, Department of Dermatology, Zhujiang Hospital, The First Military Medical University, Guangzhou, P.R.China

Cytokines are known to have an interaction with immune complexes and may be involved in the pathogenesis of cutaneous vasculitis of immune complex type. Circulating immune complexes (CIC), interleukin-1 (IL-1) and tumous necrosis factor-α (TNF-α) were assessed in sera from 16 patients with allergic cutaneous vasculitis in present study. The methods of measuring CIC, IL-1 and TNF-α were respectively anti-complement$_3$ enzyme-linked immunosorbent assay, bioassay using the 3H/HeJ mouse thymoma cell line and L929 cell line cytotoxicity assay. The results showed that serum levels of CIC, IL-1 and TNF-α in the patients were markedly increased. Significant positive correlation between CIC and IL-1 was found. The level of TNF-α in the patients with leg ulcers was higer significantly than that in the patients without leg ulcers. The results suggest interactions between CIC and cytokines may play an important role in developing of cutaneous vasculitis. Skin ulcer of this disease is due at least partly to excessive production of TNF.

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BINDING OF NEUTROPHILS TO DERMAL MICROVASCULAR ENDOTHELIAL CELLS IN VITRO:MODEL FOR EARLY STAGES OF CUTANEOUS LEUKOCYTOCLASTIC VASCULITIS Ji-Chen Ho,Marvin A.Karasek,Dept. of Dermatology,Chang Gung Memorial Hospital,Kaohsiung,Taiwan.Dept. of Dermatology, Stanford University Medical Center,Stanford, CA.

The mechanisms underlying neutrophil-endothelial cell interactions and corticosteroid inhibition in leukocytoclastic vasculits are under intense investigation. We try to isolated human dermal microvascular endothelial cells(DMEC) from normal facial skin tissue, then isolated polymorphonuclear leukocytes(PMNs) from normal human venous blood and labeled PMNs with fluorochrome. We performed neutrophil adherence assay by coincubating PMNs and DMEC in different conditions then counted adhesive PMNs with automated fluorescence analyzer.

Pretreat of DMEC monolayers with interleukin-1, TNF-alpha and lipopolysaccharide-stimulated monocyte conditioned medium result in significant increases in the adhesion of PMNs. When H-7,dexamethasone and chlorpromazine were added respectively to adherence assay, only H-7 suppressed PMNs binding significantly.

Although steroid is effective in treatment of leukocytoclastic vasculitis, we have no evidence that dexamethasone inhibits adhesivity of DMEC in the present study. Our experience support the possibility that agents which can inhibit protein kinase A and C may provide a new approach of management.

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ANTI-RANTES IMMUNOREACTIVITY IN SKIN. Petr Arenberger, Peter Nelson*, Anil Abraham** and Richard O. Leder**, Dept. of Dermatology, Charles University, Prague, Czech Republic, *Dept. of Pediatrics, Stanford University, Palo Alto, USA, and **Psoriasis Research Institute, Palo Alto, USA

RANTES shows chemoattraction of lymphocyte subsets and monocytes but expression of this cytokine in skin has not previously been demonstrated.

Cryoprepared foreskin sections were fixed and incubated with mouse anti-RANTES monoclonal antibody and FITC labeled IgG goat-anti mouse antibody. In order to demonstrate the specificity of the staining, similar procedures were performed after incubation without addition of anti-RANTES antibody. The stringent in situ hybridization was performed in the presence of the molecular probe in cryoprepared skin slices treated with formamide.

The immunoreactivity of anti-RANTES monoclonal antibody was most prominent in the str. granulosum and upper str. spinosum of normal neonatal foreskin compared with weak diffuse nonspecific FITC binding in negative controls in which anti-RANTES antibody was absent. The in situ hybridization showed the same distribution manner.

It is the first time that RANTES has been demonstrated in the skin. Previous experiments used either TNF-α or IL-1α to induce RANTES expression in human tissues. However, the foreskin appears to express the protein without any such activation. RANTES could be a marker of differenciation in epidermis due to its distribution in upper vital layers.

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SERUM FACTORS AS MARKERS OF MELANOMA PROGRESSION. Hubert Pehamberger, Roland Dorffner, Michael Binder, Robert Strohal, Klaus Wolff, Georg Stingl, Department of Dermatology, University of Vienna Medical School, Vienna, Austria

The development of metastases in approximately 20 % of melanoma patients requires a rigorous follow up. Recents studies indicate that patients with advanced tumors may exhibit elevated serum levels of certain cytokines/cytokine receptors [e.g. TNF-(R), IL-2-(R)], adhesion molecules (e.g. CD8, ICAM-1) and neoantigens (TPS). In this study we determined the serum levels of soluble (s)-TNF-R, s-IL-2-R, s-CD8, s-ICAM-1 and TPS in 177 malignant melanoma (MM) patients by ELISA and by immunoradiometric assay, respectively, and studied their correlation with tumor expression and progression. Serum factors were determined in patients (A) prior to surgery of the primary MM, (B) after surgery in tumor free follow up patients, (C) tumor free patients receiving adjuvant Interferon alpha (IFN) therapy and (D) patients with lymphnode and/or visceral metastases. The mean serum levels of s-ICAM-1, s-TNF-R, s-DC8 and s-IL2-R were found to be significantly elevated in patients with metastatic disease as compared to patients presenting with primary MM in situ without evidence of metastases or disease free follow up patients. There was no significant influence of adjuvant IFN--therapy on the serum level of the factors tested. In addition, we could not find a correlation between TPA levels and disease progression in malignant melanoma. Our study indicates that s-ICAM-1, s-TNF-R, s-CD8 and s-IL2-R are correlated with tumor growth and may be of diagnostic value for determination of metastatic progression in MM.

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TREATMENT OF CUTANEOUS LUPUS ERYTHEMATOSUS WITH A CHIMERIC MONOCLONAL CD4 ANTIBODY. J.C.Prinz*, M.Meurer*, C.Reiter*, G.Plewig*, and G.Riethmüller*, Dept. of Dermatology* and Inst. for Immunology*, University of Munich, 80337 Munich, Germany.

CD4* helper T lymphocytes are critical in the pathogenesis of lupus erythematosus (LE). Since CD4 antibodies can act as T cell selective immunosuppressive agents we have employed a chimeric recombinant human/mouse IgG1 CD4 antibody, cM-T412 (Centocor Inc., Malvern, PA), for the treatment of five patients with cutaneous manifestations of LE classified as CDLE, (n=2), SCLE, (n=1), or as SLE (n=2). A total dose of 275, 400 or 475mg antibody was administered in two cycles of 7 and 4 daily intraveneous infusions at an intervall of 4-6 weeks. In the SLE patients, antibody was combined with internal steroids.

In all patients, cutaneous inflammatory lesions cleared shortly after antibody application. In four patients the disease took a more moderate course, with permanent healing or a better response to conventional treatment of the majority of skin lesions and, in one SLE patient, lasting improvement of a nephrotic syndrom as a longterm effect. The other SLE patient, however, showed an early relapse. Anti-CD4 treatment resulted in a distinct reduction of circulating helper T lymphocytes, yet without clinical signs of immunosuppression. Immunohistochemical analysis of biopsies taken from lesional skin before and after anti-CD4 treatment showed a reduction of infiltrating CD4* T cells and of the expression of cellular IL-2-receptor, ICAM 1 and MHC class II. In summary these results indicate that blocking of helper T cell functions by CD4 antibodies may help to control the mechanisms leading to tissue damage of cutaneous LE lesions.

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S-PHASE LYMPHOCYTES OF PERIPHERAL BLOOD IN PATIENTS WITH AUTOSENSITIZATION DERMATITIS. Kazuo Sasaki and Masami Uehara, Department of Dermatology, Shiga University of Medical Science, Otsu, Japan

In 12 patients with autosensitization dermatitis, we examined the number of S-phase peripheral blood lymphocytes at the time of acute exacerbation and after treatment. Fourteen healthy volunteers served as normal controls. Peripheral blood lymphocytes were suspended at 2 x 10^6 lymphocytes/mL of a culture medium, and then 10 µmol bromodeoxyuridine (BrdU) was added. Cultures were incubated for 30 min. After completing the cultures, the cells were fixed, and denatured of the cellular DNA. Then, the cells were incubated with FITC-conjugated monoclonal anti-BrdU antibody. The staining of DNA was performed in 5 µg/mL of propidium iodide. Flow cytometry was performed on 10,000 cells from each sample.

In normal controls, the percentage of $BrdU^+$ cells (S-phase lymphocytes) were 0.39 ± 0.35%. In patients with autosensitization dermatitis, the proportion of $BrdU^+$ cells were 4.66 ± 3.33% at the time of acute exacerbation of skin lesions, and 0.87 ± 0.58% after treatment. This difference was significant ($P < 0.05$). These findings suggest that at the time of exacerbation of dermatitis, there may be circulating antigen(s) which activate peripheral lymphocytes of patients with the skin disease.

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A SERUM FACTOR IN SLE PATIENTS WHICH MODULATE CD4 AND CD45RA ANTIGENS ON THE SURFACE OF CIRCULATING T LYMPHOCYTES. Toshiko Nobutoh, Mamoru Kohda, Hiroaki Ueki, Department of Dermatology, Kawasaki Medical School, Kurashiki, Japan

Decreased CD4+CD45RA+ lymphocytes have been reported in peripheral blood of SLE patients. In this study, mononuclear cells were separated from peripheral blood of SLE, and the CD4+CD45RA+ cells were counted by flow cytometry at the day and one week later in order to investigate whether these decreased cells could be initiated by their own intracellular natures or by some extrinsic factors. Furthermore healthy lymphocytes were incubated with SLE patient's sera and Ca++ level were counted. The CD4+CD45RA+ lymphocytes decreased at the first day just after separation, but recovered to normal range after one week culture without patient's sera. The Ca++ uptake of normal lymphocytes increased one minute after incubation with patient's sera, but not with the sera of normal control. These results suggested that CD4+CD45RA+ cells were persistently activated in the peripheral blood of SLE patients, and that their sera contained some extrinsic factors which could activate the lymphocytes.

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IMMUNOHISTOLOGICAL DETECTION OF RETINOID-X RECEPTOR (RXR–α,–β, - γ) EXPRESSION IN NORMAL AND PSORIATIC HUMAN SKIN

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Recently, is has been shown that retinoid acid receptors (RAR –α, –β, –γ) and vitamin D receptor (VDR) require auxiliary proteins for effectice DNA-binding to their responsive elements in target genes, thus regulating transcriptional activity. These proteins were identified as the retinoid-X receptors (RXR), forming heterodimeric complexes with several steroid hormone receptors including RARs and VDR. While RARs are activated by all trans retinoic acid (t-RA) as well as by 9-cis retinoic acid (9-cis RA), RXR were found to bind only to 9-cis RA with high affinity. We now investigated RXR-α,–β,–γ expression in normal and psoriatic human skin *in situ*, applying antibodies against the different RXR-types and an immunoperoxidase technique. RXR-positive cells related to the skin immune system were immunophenotyped on sequential sections by a double-labeling procedure for the simultaneous demonstration of nuclear receptors and cluster definded (CD) cell membrane antigens as well as cytokeratin, vimentin and S-100 protein. Our findings indicate that (I) RXR-α and -γ are strongly expressed in normal and psoriatic human skin, in contrast to RXR-β, (II) both the subepidermal and subcellular distribution of RXR-α and -γ point to a function for the switch from proliferation to differentiation in epidermal keratinocytes; (III) RXR-α and -γ expression seems to be modulated in skin diseases characterized by altered epidermal differentiation, such as psoriasis; and (IV) RXR-α and -γ are expressed in various cell types related to the skin immune system, e.g. dendritic epidermal cells, indicating that 9-cis RA might not only be acting on an altered epidermal differentiation, but also might be a potent immunomodulating drug.

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UPREGULATION OF 1,25-DIHYDROXYVITAMIN D3-RECEPTOR (VDR) EXPRESSION, AND MODULATION OF MARKERS FOR PROLIFERATION, DIFFERENTIATION AND INFLAMMATION IN CALCIPOTRIOL (MC 903) TREATED PSORIATIC SKIN. [1]Epple S., [1]Bahmer FA, [1]Kerber A, [2]Kragballe K, [3]Rauterberg EW, [1]Reichrath J, [1]Dept. of Dermatology, University of the Saarland, Homburg, [2]Dept. of Dermatology, Marselisborg Hospital, University of Aarhus, Denmark, [3]University of Heidelberg, Germany.

Vitamin D and analogues have been successfully used for the topical treatment of Psoriasis vulgaris. Besides its ability to suppress the proliferation and to induce the differentiation of keratinocytes in vitro, 1,25-dihydroxyvitamin D3 and analogues were shown to act on various cell types related to the skin immune system, that express VDR. Furthermore, the production of various cytokines such as IL-8 is modulated in numerous target cells by this potent steroid hormone. We investigated immunohistochemically the changes in 1,25-dihydroxyvitamin D3 receptor (VDR) expression, inflammation, proliferation and differentiation during Calcipotriol (MC 903) treatment in situ. Biopsies were taken at days 0, 7 and 42 from lesional and nonlesional psoriatic skin and snap frozen in liquid nitrogen and stored at -70°C. On cryostat sections, VDR expression was analyzed immunohistochemically applying the monoclonal antibodies 9A7γ and VD2. Changes in inflammation were investigated by Abs against CD 1a, 4, 8, 11b, 15, NAP-1/IL-8, 55 kDa TNF-Rpt., ICAM, HLA-DR. Changes in differentiation were analyzed by MoAbs against involucrin, transglutaminase K and keratin 16. Proliferation was investigated by MoAbs Ki-67 and against PCNA. For morphometric analysis, different horizontal compartments were defined in each skin section as follows: epidermis, upper dermis/perivascular loop, lower dermis/perivascular superficial plexus. We found a significantly increased VDR expression of epidermal basal keratinocytes in lesional psoriatic skin along with topical Calcipotriol treatment. A considerable 50% decrease of the volume density of proliferating epidermal cells, measured as Ki-67 positive nuclear volume was found after 6 weeks while the distribution and intensity of keratin 16, transglutaminase and involucrin immunoreactivity decreased almost completely to the appearance for clinically uninvolved psoriatic skin. After six weeks of topical calcipotriol treatment the immunoreactivity of antibodies against CD 4, 8, 11b, 15 in the epidermis changed nearly to the pattern characteristic for nonlesional psoriatic skin, while a large number of these markers remained to be in the dermal compartments. Our findings indicate (i) that topical treatment with MC 903 increases significantly VDR expression in epidermal basal cells in lesional psoriatic skin; (ii) differentiation and proliferation of epidermal cells seems to be stronger affected than inflammation; (iii) MC 903 exerts a specific effect on inflammation in vivo, probably via VDR and the modulation of cytokine production in skin immune cells.

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RECONSTRUCTION OF PSORIATIC EPIDERMIS FOLLOWED BY T CELL HYPERPROLIFERATION IN TRANSPLANTED PSORIASIS SKIN IN SCID MICE. Tsukasa Ohkawa, Takao Fujimura, Yuko Hamada, Hikaru Eto, Mikio Masuzawa and Shigeo Nishiyama, Department of Dermatology, Kitasato University School of Medicine, Sagamihara, Kanagawa, Japan

Investigation into the pathogenesis of psoriasis have been disturbed by the lack of adequate animal model. In the present study, we analyzed transplanted psoriasis lesional skin in SCID mice which lack of functional lymphocyte. Grafted lesional skins were sequentially biopsied every 2 weeks after graft, and examined histologically and immunohistologically. The expression of interferon-gamma mRNA was also analyzed by RT-PCR method. All grafted lesional skins were maintained without rejection. Psoriatic histological feature graduately disappeared in correlation with the decrease of CD3 positive human cells. Expression of interferon-gamma mRNA also disappeared when psoriatic histological feature were normalized. However, we found a case with CD4 positive human cell hyperproliferation at 6 weeks after graft. Interestingly, in this case, Psoriatic epidermal histological feature reconstructed in parallel with expression of interferon-gamma mRNA. These findings suggested that the psoriatic epidermal hyperproliferation was induced by interferon-gamma producing T cell subset

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ANTIBODY TO 65-KD HEAT-SHOCK PROTEIN (HSP65) IN PSORIASIS, PALMOPLANTAR PUSTULOSIS AND INFLAMMATORY SKIN DISEASES Y. Goto[1], S.Izaki[1], K. Kitamura[1], and H. Nomaguchi[2], [1]Department of Dermatology, Saitama Medical Center, Kawagoe, and [2]National Institute for Leprosy Research, Higashi-Murayama, Japan

Cells from procaryotes and eucaryotes exposed to environmental change produce a series of proteins called "heat shock protein" (HSP), or stress protein, the amino acid sequence of that has been highly conserved. Recent investigators suggest that the reaction to the shared antigen between HSP's may account for induction and modification of inflammation, as well as some autoimmune mechanism. In the present study, antibody level to HSP65 of Mycobacterium leprae was investigated with enzyme-linked immunosorbent assay (ELISA) in various skin diseases including psoriasis and palmoplantar pustulosis. From totally 200 patients, normal cases (n=9) including patients with nevocellular nevus showed 0.096 ± 0.036 (mean ± SD) in IgG level (OD492). Among patients with psoriasis 7 cases out of 8 tested demonstrated elevated anti-HSP65 exceeding normal mean ± 1 SD level in IgG, and patients with PPP showed 14 cases out of 20 tested. All patients judged as focal infection-related (n=4) showed significantly high level of anti-HSP65. Herpes zoster (n=13) and urticaria (n=29) showed similarly elevated anti-HSP65 antibody. Anaphylactoid purpura showed limited rate (2 cases out of 5 tested) in elevation of anti-HSP65. Similar tendency was found in anti-HSP65 IgM level but not in IgA. In conclusion, anti-HSP65 antibody may be significant in certain focal infection as observed in cases with psoriasis and PPP, while some other focal infection-related disease such as anaphylactoid purpura did not always show this antibody elevation. In addition, viral infection should be included in antibody production to HSP65. Further studies are required to clarify relationship between bacterial and viral infection and inflammatory skin diseases.

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ENHANCING EFFECT OF STAPHYLOCOCCAL ADHESINS ON THE INTERACTION BETWEEN CORNEOCYTES AND NEUTROPHILS. Taizo Kato, Tadashi Terui and Hachiro Tagami, Department of Dermatology, Tohoku University School of Medicine, Sendai, Japan

Stratum corneum (SC) activates complement to generate a chemotactic C5a. Opsonic componetnts C3b or C3bi are also produced in this process. Neutrophils accumulated by C5a at the subcorneal portion are expected to interact the opsonized SC in a form of frustrated phagocytosis.

We studied the effect of staphylococcal protein A (PA) and lipoteichoic acid (LTA), which are nominated as the adhesin of Staphylococcus aureus (S. aureus), on the frustrated phagocytosis assessed by the binding assasy of neutrophils on the SC as well as SC-induced chemiluminescnce (CL) in neutrophils. We found that PA and LTA significantly promoted opsonized SC-induced CL in neutrophils. It took place even with the serum obtained from a patient with agammaglobulinemia. Microscopic observation of such SC revealed an increase in the number of neutrophils adhering to the SC surface of the adhesin-coated corneocytes. The binding of neutrophils on the SC surface was almost completely suppressed by the blocking assay with anti-CR3 (CD11b) antibody to neutrophils.

Our hypotehsis is that S. aureus increases the number of neutrophils specificially attaching to the surface of opsonized SC mainly through the CR3 receptor of neutrophils, resulting in an enhanced respiratory burst of neutrophils. The resultant extracellular release of active oxygen species may cause damage to the epdiermal tissue surrounding S. aureus, which has colonized on the SC and escaped from phagocytosis.

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INTERACTION OF *STAPHYLOCOCCUS AUREUS* CELLS AND SILK THREADS IN VITRO AND IN MOUSE SKIN. Hisanori Akiyama, Rikako Torigoe and Jirô Arata, Department of Dermatology, Okayama University Medical School, Okayama, Japan

Staphylococcus aureus cell suspension was epicutaneously inoculated on the back skin of cyclophosphamide-treated mice with silk stitches and these sites were occluded. Biopsy specimens were taken from three mice at 1, 3, 6, 12, 24, 48, and 72 h after inoculation and were examined by electron microscopy. Fibril-like structures were seen around the *S. aureus* cells at 1 h. At 3 h, they had extended towards the silk threads. There were microcolonies on the surfaces of the silk threads and, at 12h the *S. aureus* cells were enclosed in membrane-like structures. The electron density of the membrane-like structures increased over time. After ruthenium red staining, the membrane-like structures and the fibril-like structures were stained positive, suggesting that these structures contain polysaccharide components. With a combination chemotherapy using clarithromycin and ofloxacin, *S. aureus* cells in the membrane-like structures were degenerated, whereas the use of clarithromycin or ofloxacin alone had little effect. Chlorhexidin gluconate and povidone iodine were effective if they were able to reach the biofilm. The fibril-like structures appeared in vitro only in the presence of silk threads, and were enhanced by the presence of mouse plasma. These structures did not form with formaldehyde-killed *S. aureus* cells. Thus, *S. aureus* cells may interact with foreign bodies to form biofilms, thereby evading the effect of antibacterial agents, and prolonging infection.

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CHANGES IN THE CUTANEOUS INFLAMMATORY INFILTRATE DURING TREATMENT OF ATOPIC DERMATITIS WITH CYCLOSPORINE. **J Berth-Jones, A Fletcher,* RAC Graham-Brown, Departments of Dermatology and *Histopathology, Leicester Royal Infirmary, Leicester, UK.**

Cyclosporine is an effective treatment for atopic dermatitis (AD). To investigate the mechanism of action components of the epidermal and dermal inflammatory infiltrate were assessed during a placebo-controlled, crossover trial. Eight adult patients with severe AD received cyclosporine 5mg/kg/day and placebo, each for 8 weeks. Six mm punch biopsies were obtained from lesional skin at baseline, and at the end of each treatment period. Sections were stained with H&E and a mast cell stain (CASABA technique), and labelled with monoclonal antibodies to HLADR, CD1a, CD4, CD8, CD25 and a macrophage marker (PGM1).

There was a clear reduction in epidermal spongiosis and acanthosis; the reduction in density of the dermal lymphohistiocytic infiltrate was less pronounced. There were clear and consistent reductions in expression of CD25 and in macrophage infiltration (in both epidermal and dermal compartments). Reductions in lymphocyte counts, HLADR expression and CD1a+ve dendritic cells were less consistent. There was no apparent effect on the number of dermal mast cells.

The data suggest the response to cyclosporine is linked to reduced lymphocyte activation and reduced macrophage chemotaxis.

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SOLUBLE INTERCELLULAR ADHESION MOLECULE-1(sICAM-1) IN SERA OF PATIENTS WITH ATOPIC DERMATITIS AND PSORIASIS. Masayo Koide, Yoshiki Tokura, Fukumi Furukawa, Masahiro Takigawa, Department of Dermatology, Hamamatsu Red Cross Hospital, Department of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, Japan

Intercellular adhesion molecule-1(ICAM-1), a member of the immunoglobulin supergene family with five- domain structure, is known to play an important role in various inflammatory diseases. We determined levels of soluble ICAM-1 (sICAM-1) in sera from patients with atopic dermatitis (AD), psoriasis and other inflammatory cutaneous diseases as well as normal individuals. In AD patients sICAM-1 levels were significantly higher than normal control and improvement of skin lesions was correlated with a decrease in sICAM-1 levels. In psoriasis the levels of were proportional to the PASI score and dependent on the disease activity. These findings suggested that the determination of sICAM-1 level provided an impotant mean for monitoring the activity of inflammatory skin diseases.

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HOUSE DUST MITE ANTIGEN INDUCED Fcε RECEPTOR II(CD23) EXPRESSION IN MITE-SENSITIZED ATOPIC DERMATITIS PATIENTS. Zhang Hui-min, Keisuke Maeda, Yoichi Tanaka, Hikotaro Yoshida, Department of Dermatology, Nagasaki University School of Medicine, Nagasaki, Japan

Recently, major interest has been addressed to the role of the Fcε receptor II(CD23) in atopic dermatitis (AD). On peripheral blood mononuclear cells (PBMC) of patients with AD, CD23 expression can be enhanced by allergen stimulation. The aim of this work is to analyse CD23 expression of AD after stimulation with affinity-purified house dust mite antigen.

We studied 15 mite-sensitized AD patients by patch test and RAST for Dermatophagoides farinae (DF), 5 mite-nonsensitized AD patients and 10 healthy non-atopic volunteers. PBMC were isolated by centrifugation on a Ficoll-paque gradient. PBMC suspensions($1x10^6$/ml) were cultured in RPMI 1640 medium with affinity-purified DF extract (20μg/ml) for up to 7 days. In order to determine the subset of $CD23^+$ PBMC, we carried out double-labelling experiments with FITC-conjugated CD23 (Immunotech S.A.) followed by incubation with PE-conjugated antibodies Leu 16 (B cells), CD14 (monocytes/macrophages), CD4 (helper T cells) or CD8 (suppressor T cells). The fluorescence intensity of 20,000 live cells were analysed on a Beckton Dickinson FACScan flow cytometer.

Treatment of PBMC from mite-sensitized AD patients with DF extract induced maximal expression of CD23 on day 3 of culture. The frequencies of $CD23^+$ PBMC after stimulation with DF extract were significantly higher than the cells without stimulation as control. Furthermore, the whole PBMC subset showed an increased expression of CD23. No significant increase in frequency of $CD23^+$ PBMC was found in non-sensitized AD patients and healthy controls.

The results demonstrate that CD23 expression on PBMC can be enhanced by appropriate allergen stimulation in AD. The increase of CD23 levels may modulate the IgE-dependent inflammation in lesions of AD.

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IGE-RAST STUDY ON ALLERGENIC CROSS-REACTIVITY OF COOKS-FOOT POLLEN AND CEREAL PROTEINS SUCH AS RICE AND WHEAT USING SERA FROM PATIENTS WITH ATOPIC DERMATITIS. K.Tubaki*, K.Miyakawa, C.Suga, H.Komatsu, A.Sugiyama and Z.Ikezawa, Dep.Dermatol., Yokohama City Uni. School of Medicine, Urafune Hospital, Yokohama, Japan, *Asahidenka-Kogyo Institute, Tokyo, Japan.

Recently, patients with severe type of atopic dermatitis(AD) are increasing in Japan. In these patients, serum IgE value and frequency of RAST-positive allergens are high and the correlation coefficient betweenthe both data is high (γ =0.712; n=948, $p<0.01$), suggesting that the RAST titers for several allergens mutually correlate. Then we calculated the correlation coefficient among the RAST titers for many allergens, and there turned out to be a very close correlationship between mite and house dust, among cereals such as rice, wheat, soy bean, peanut, buckwheat, corn, sesame, fox tail millet and barnyard grass, between these cereals and cooks foot pollen, and among fungi such as candida, clado-sporium and altenaria. The RAST inhibition studies revealed there is a cross-reaction not only among cereals but also between these cereals andcooks foot pollen. Therefore, it is presumed that the croos-reaction of IgE antibodies to the both cereal foods and grass pollens may act on the breakdown of oral tolerance to the cereal food allergy in severe AD. Also allergenic cross-hypersensitivity to both grass pollen and wheat proteins in some cases of perenial rhinitis and asthma is suggested by the observation that symptomes improve when wheat products are eliminated from the diet.

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EOSINOPHIL CHEMILUMINESCENCE RESPONSES TO CYTOKINES AND OPSONIZED ZYMOSANS IN ATOPIC DERMATITIS. Kano Taguchi, Minoru Miyasato, Keiko Kato, Shingo Tsuda and Yoichiro Sasai, Department of Dermatology, Kurume University School of Medicine, Kurume, Japan

Our previous observation demonstrated a positive correlation among the grading severity of atopic dermatitis (AD), peripheral blood eosinophil count and serum level of eosinophil cationic protein (ECP) as a reflection of eosinophil activation in vivo. To extend this observation, comparison of eosinophil responsiveness to cytokines and opsonized zymosans was done between clinically graded AD and healthy subjects. Blood eosinophils (Eos) were isolated by an anti-CD16/ immunobeads/ Nycodenz density gradient combination method. Functional responses of Eos were measured by MCLA- and luminol-chemiluminescence (CL) to IL-3, GM-CSF, IL-5, C_3-coated zymosan and IgG-coated zymosan (IgGZ). Upon activation with these stimuli, Eos from AD patients usually elabolated higher MCLA- and luminol-CL than Eos from healthy subjects. Statistical analysis revealed a good correlation between the grading severity of AD and only CLs responses of Eos to IL-5 and IgGZ. There was a linear relationship between serum level of ECP and these two stimuli-induced integral CLs intensities. Additionally, flow cytometric evaluation showed that the expression of FcrRII (CD32) on Eos not only correlated with clinical severity of AD, but also intensity of IgGZ-induced CLs depended on the proportion of CD32-positive Eos. We also demonstrated the presence of a specific IgG antibody against mite antigen in sera from all patients with AD. These results suggested a possibility that increased serum level of ECP in AD patients might be due to eosinophil activation induced by IL-5 and IgG-antigen immune complexes in vivo.

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RESPONSES TO MITE ANTIGEN AND SUPERANTIGENS OF PERIPHERAL BLOOD MONONUCLEAR CELLS IN PATIENTS WITH ATOPIC DERMATITIS. Ryuichi Yokote, Yoshiki Tokura, Hiroaki Yagi, and Masahiro Takigawa, Department of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, Japan

The relationship between the in vitro proliferative responses of peripheral blood mononuclear cells (PBMC) to mite antigen (MAg) and to superantigens (sAgs) was examined in patients with atopic dermatitis. PBMC from some atopic patients responded well to MAg. Irrespective of the degree of responsiveness to MAg, there was no significant difference in the responses to SEB and TSST-1 among patients with atopic dermatitis and normal subjects. To address the issue that T cell populations that react to MAg were proliferative in response to the further addition of sAg, correlation between the stimulation index of MAg and that of MAg plus sAg (MAg/sAg) was elucidated. To eliminate the simple additional effect of MAg in the T cell response induced by MAg/sAg, the proliferative response to MAg/sAg was expressed by subtracting the response to MAg alone. The responsiveness of PBMC to MAg/sAg was correlated to the magnitude of response induced by MAg alone in individual patients with atopic dermatitis. Thus, there was a synergistic effect between MAg and sAg in the stimulation of MAg-reactive T cells. The in vitro production of IL-4 by PBMC from atopic patients was increased by culturing cells with MAg/sAg. These findings suggested that the majority of T cell populations reactive to MAg are capable of proliferating in response to sAg.

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PREPARATION OF HYPOALLERGENIC WHEAT FOR PATIENTS WITH ATOPIC DERMATITIS,WHO WERE SUSPECTED WHEAT ALLERGY, AND ITS CLINICAL APPLICATION. Z.Ikezawa, K. Miyakawa, C.Suga, H.Komatsu, A.Sugiyama and K.Tsubaki*, Dep.Dermatol., Yokohama City Uni. School of Medicine, Urafune Hospital, Yokohama, Japan, Asahidenka-Kogyo Institute, Tokyo, Japan.

We have already succeeded in preparation of hypoallergenic rice for elimination diet of rice in the patients of severe atopic dermatitis(AD)with rice allergy. We also revealed that there are partial cross-reactions between rice and wheat antigens, and that wheat allergy may play an important role in developement of severe AD in some cases. The analysis of wheat antigen by IgG-, IgA- or IgE-immunoblotting revealed that the low molecular proteins specifically recognized with the IgG antibody from AD patient's sera also reacted with the IgE, but not with the IgA. On the contrary the IgG-immunobolting pattern with normal sera is the same as the IgA-pattern. Also we fractionated the wheat antigen to water-, 1M NaCl-, 70% ethanol-and 4M urea-soluble fractions, and assayed IgE antibody titer against each fraction. The results indicated that salt-soluble fraction contained most of low molecular ones and might be majorallergenic component of wheat antigen. Then we prepared hypoallergenic wheat by eliminating the water- and salt-soluble fractions from the original wheat, and examined its clinical effect on atopic dermatitis with suspected wheat allergy.

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EFFECT OF ORAL ANTI-FUNGAL DRUGS ON RECALCITRANT ATOPIC DERMATITIS. Chizuka Suga, Yukari Yamamoto, Kanata Miyakawa, Hitoshi Komatsu, Kazuko Kitamura and Zenro Ikezawa, Dep. Dermatol., Yokohama City Uni. School of Medicine, Urafune Hospital, Yokohama, Japan

Matsuda et al have reported remarkable effectiveness of anti-fungal nystatin on severe atopic dermatitis(AD) as one of evidences suggesting Yeast Connection. We have also reported that there is a strong correlation between severity of AD and IgE antibody titers to candida. Then, we invesigated a relationship between the clinical effectiveness of anti-fungal drugs and the serum IgE antibody titers to candida, candida detection in culture of stools and scores of questionnaires about Yeast Connection performed in 76 recalcitrant AD patients, who were subgrouped to two groups with and without elimination diet and orally given anti-fungal nystatin and amphotericin B. The clinical improvement was observed more frequently in the group with than without elimination diet, in the group with RAST-food(-)/-candida(+) than with the both RAST(+), and in the group with positive than negative in the candida stool detection. Also the serum IgE antibody titers to candida and scores of the questionnaires were higher in the group with improvment effect than in the other. These results indicate a probable involvement of intestinal candidiasis in development of recalcitrant AD.

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ORAL ADMINISTRATION OF PIROXICAM WITH UVA IRRADIATION BEFORE SENSITIZATION INDUCED A CROSS-TOLERANCE TO NOT ONLY THIMEROSAL/THIOSAIICYLATE-CONTACT HYPERSENSITIVITY BUT ALSO TENOXICAM-PHOTOSENSITIVITY IN GUINEA PIGS. Junko Osawa, Takeshi Hariya*, Kazuko Kitamura and Zenro Ikezawa, Department of Dermatology, Yokohama City University Urafune Hospital, *Shiseido Safety & Analytical Research Center, Yokohama, Japan

Piroxicam(PXM) is well known as an anti-inflammatory drug, which causes photosensitive eruptions. Recently, we revealed that there is a cross-reaction between photosensitivity to PXM and tenoxicam(TXM), which is the same oxicam-family drug as PXM, as well as between photosensitivity to PXM and contact hypersenstivity to thimerosal(TMS) and thiosalicylate(TOS) in guinea pigs, and that the cross-reacting epitope between PXM- and TXM-photosensitivity is different from that between PXM-photosensitivity and TMS/TOS-contact hypersenstivity. Then, we examined the cross-tolerance among PXM- and TXM-photosensitivity and TOS-contact hypersenstivity. The results demonstrated that oral administration of PXM with UVA irradiation before sensitization resulted in a cross-tolerance to TMS/TOS-contact hypersensitivity and TXM-photosensitivity induced in guinea pigs photocontact sensitzed with PXM, and also did in a specific tolerance to PXM-photosensitivity induced in animals photo-contact sensitized TXM, as well as in animals contact sensitized with TOS.

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HYDRATION DERMATITIS. OCCLUSION ALONE RAPIDLY INJURES HUMAN SKIN. Tracy Stoudemayer, Peishu Zheng, Albert M. Kligman, S.K.I.N. Inc. and Department of Dermatology, University of Pennsylvania, Philadelphia, PA, USA

Prolonged exposure to water may cause a dermatitis. This is often ascribed to surfactants (housewives, hairdressers) or microbial infections (soldiers). Occlusion is also used to enhance penetration of drugs and in patch testing.

We applied water soaked patches occlusively to human skin for periods from 2 days to 3 weeks. After removal of the patches clinical observations were made and punch biopsies were obtained. We observed histologic damage in as early as two days at which time cytologic changes were observed by TEM in Langerhans cells and keratinocytes. Subsequently, a brisk inflammation reaction developed with infiltration of leukocytes and damage to vascular endothelium.

After continuous hydration for two to three weeks, the skin appeared normal (except for some maceration) but showed a "rebound" dermatitis 2 to 3 days after. Suppression of microorganisms during the exposure did not prevent the dermatitis.

We postulate the release of pro-inflammatory cytokines, such as interleukin I, from the swollen horny layer. These observations have relevance to the interpretation of occlusive patch testing, to the use of occlusion to enhance penetration of drugs and to eczematous reactions among wet workers (housewives, hairdressers etc.).

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INHIBITION OF CONTACT HYPERSENSITIVITY BY AN ANTI-ALLERGIC DRUG, AZELASTINE, IS LIKELY TO BE MEDIATED BY ITS NOVEL IMMUNOSUPPRESSIVE EFFECTS DISTINCT FROM FK-506. Masutaka Furue, Atsushi Osada, and Kunihiko Tamaki. Department of Dermatology, Yamanashi Medical University, Yamanashi, Japan

Azelastine hydrochloride (AZE) is a phthalazinone derivative which is now widely used in Japan for the treatment of atopic disorders as an effective long-acting anti-allergic drug. The purpose of the present study is first to characterize the immunosuppressive activity of AZE in vitro in comparison with very powerful, well-characterized immunosuppressant, FK-506, and secondly to investigate whether topical application of AZE has a capacity to inhibit the contact hypersensitivity in vivo. We compared the immunosuppressive effects of AZE and FK-506 on the murine splenocytes stimulated by Con A, and found that 1) AZE inhibited the [^{3}H]-TdR incorporation in a dose-dependent fashion (ED_{50}= 3.3×10^{-6}M), 2) the suppression of IL-2 production by AZE was limited and considerably large amounts of IL-2 were still produced even in the presence of 10^{-5}M of AZE, which was in sharp contrast to the marked inhibition of [^{3}H]-TdR incorporation, 3) the inhibitory action was significantly restored by Rat-T-STIM (a mixture of cytokines), but not by other recombinant cytokines examined, 4) the significant inhibitory action was still observed even when AZE was added at 48hr after the initiation of culture, 5) AZE inhibited the Con A-induced cluster formation of splenocytes. With regards to FK-506, the immunosuppressive mechanism(s) was likely to be due to the inhibition of IL-2 production, because 1) FK-506 completely blocked the production of IL-2, 2) exogeneous IL-2 consistently restored the FK-506-induced inhibition, 3) the significant suppression was observed only when FK-506 was added within 24hr after the initiation of culture. Thus, in vitro immunosuppressive effect of AZE was clearly different from that of FK-506. Moreover, the single topical application of 3.3% of AZE (0.08M) strongly inhibited the efferent phase of contact hypersensitivity to TNCB as topical steroid did, suggesting that the immunosuppressive activity shown in vitro may be operative in vivo. Phthalazinone derivative(s) is a potential immunosuppressive agent which may be therapeutically valuable in topical use.

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BA-MCAB-ELISA AND MCAB-ELISA FOR THE DETECTION OF HUMAN SERUM PENICILLIN-SPECIFIC-IgE AND ITS CLINICAL EVALUATION. Shi-yin Li and Yong-sheng Liu, Department of Dermatology, Beijing Medical University, Beijing, P.R.China

The research of penicillin allergy needs the assay for the detection of penicillin specific IgE to be of highly accuracy and great convenience. Using monoclonal antibodies(McAb) and biotin- avidin system (BAS), we developed a new method--BAS-McAb-ELISA for the detection of penicilloyl (BPO) specific IgE and McAb-ELISA for the detection of BPE/6APA-specific IgE in the sera of patients with penicillin allergy. This method was proved to be highly specific and 64 times more sensitive than the improved ELISA. In our data there was no significant interference by serum p-spec-IgG observed in the quantitation of p-spec-IgE, and the inhibition effect of p-spec-IgG on urticarial type reaction to penicillin can not be proved. The results of BPO-spec-IgE were all negative in both 201 normal students and 114 patients without penicillin allergy. In patients with positive history of penicillin allergy and penicillin skin test, the positive rate was 49%(24/49). We also detected BPE/6APA-spec-IgE in the sera of patients without BPO-spec-IgE using McAb-ELISA, its positive rates were 12.2%(6/49) and 32.7%(16/49) respectively. The total positive rate of p-spec-IgE was 93.8%(48/49). Moreover in seven cases of penicillin anaphylactic shock we found that BPO-specific and BPE/6APA specific IgE were positive in three and four cases respectively. The preliminary results showed that both minor and BPO determinant play important roles in the anaphylactic shock of penicillin allergy. We also compared BA-McAb-ELISA, BA- PcAb-ELISA, ABC-McAb-ELISA and PcAb-ELISA for the detections of human serum total IgE. We found that the BA-McAb-ELISA was more sensitive and stable than the other three methods. The value of total human serum IgE detection by BA-McAb-ELISA corresponded with values obtained by the other three ELISA.

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LANGERHANS' CELLS IN CONTACT HYPERSENSITIVITY TO PLATINOID METALLS AND ATOPIC DERMATITIS. Victor I. Prokhorenkov, Department of Dermatology and Venerology, Krasnoyarsk Medical Institute, Krasnoyarsk, Russia

Langerhans' cells (LC) performing the antigen-producing function in epidermocyte play an important part in pathogenesis of contact eczema and atopic dermatitis. There is an interest in comparative study LC function in contact allergy and atopic dermatitis.

We studied skin microbioptats in 12 workers with contact eczema induced by industrial contact with platinoids (etiologic diagnosis confirmed by the skin tests) and in 7 patients with atopic dermatitis. In chilled sections Langerhans' cells were determined by immunomorphologic method using antibodies and Ia-, T6-AG and also by W.B. Shelley, L.Juhlin (1977) and S.Sjöberg (1978) methods.

There was revealed the decrease of HLA-DR$^+$ quantity of LC in epidermis in patients with contact eczema. Intracellular contain of catecholamines was not changed reliably. In atopic dermatitis HLA-DR$^+$ quantity of LC decreased only in acute dermatosis especially in patients who received steroid ointments locally and ultraviolet irradiation. Simultaneously in patients with atopic dermatitis there was revealed a marked increase of the monocytes quantity which had HLA-DR molecules on their surface membranes up to 82,2% (in control group 64,8%).

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RETINOID-COMBINATION THERAPY FOR PSORIASIS DOWN-REGULATES INCREASED EXPRESSION OF CELL ADHESION MOLECULE BY ENDOTHELIAL CELLS Kiichiro Danno, Department of Dermatology, Shiga University of Medical Science, Otsu, Japan.

Various treatment modalities with different therapeutic mechanisms are beneficial for treatment of psoriasis. Although immunomodulatory effects of them have recently been emphasized as an important aspect of action mechanisms, effects on the cell adhesion molecule (CAM) expression by endothelial cells (ECs) have not yet been thoroughly investigated. In this study, biopsy specimens were taken from typical lesions of 23 cases before and during treatment with psoralen plus UVA (PUVA) radiation, UVB radiation, and oral eicosapentaenoic acid (EPA) alone or combined with oral etretinate (Re). Immunohistochemical techniques were applied to visualize the expression of ELAM-1 and ICAM-1 by ECs. The staining intensity of them was correlated with the histological features to determine whether alterations in the CAM expression precede or follow the normalization of the histological characteristics. Following Re-combination therapies, increased expression of both CAMs by ECs in the papillary dermis was remarkably decreased to the baseline conditions before the histological features were normalized, while a delayed or unremarkable response was observed to PUVA, UVB, and EPA alone. The preliminary results suggest that whether Re alone can directly or indirectly down-regulate the immunologic function of activated ECs, which in part plays a pathogenetic role in psoriasis, or it enhances the inhibitory effect of other single regimens on ECs.

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ELIMINATION OF IMMUNE COMPLEXES BY THE SKIN. Ludmila Beletskaya, Flora Baranova, Institute for Transplantation and Artifitial Organs, Moscow, Russia.

No immunoglobulin have been revealed in the epidermis of young healthy subjects and animals. The onset of infection (for example, appendicitis, cholecystitis e.c.) is associated with the appearance of minute immunoglobulincontaning granules in the epidermis, which is regarded as the physiological ability of the skin to eliminate immune complexes. The failure of this function in the number of diseases (systemic lupus erythematosus and other immunocomplex diseases) is accompanied by deposition of immune complex (IC) granules in the derma and in the derma-epidermal zone. IC actively involved in metabolitic processes that lead to disturbance tissue structures. Sometimes at the same disease failure of elimination function absent immune complex deposits and clinical manifestation absent too. Some treatment undertaking (for example carbo-hemoperfusion) followed by enhance of many phisiological functions including the function of skin IC elimination (Beletskaya et al.,1983). The last have accompanied by appearance of immunecomplex granules in the epidermis and on its surface. This data support supposition of immune-complex elimination skin function exist.

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CHARACTERIZATION OF A REVERSIBLE INTERMEDIATE FORMED DURING THE SPONTANEOUS INACTIVATION OF HUMAN SKIN TRYPTASE . April M. James, Grace Y. Eng, Darrell R. McCaslin, and Norman M. Schechter, University of Pennsylvania, Philadelphia, PA, Rutgers University, Newark, NJ, USA

Regulation of the human mast cell serine proteinase, tryptase, in inflammation is unclear because there is no physiological inhibitor to inactivate the secreted enzyme. The isolated proteinase rapidly inactivates when incubated in solutions of physiological salt and pH, suggesting that an auto-inactivation process may replace the need for a specific inhibitor. We have provided evidence that loss of activity involves the rapid equilibration of active tryptase with an inactive form capable of re-activation by heparin which then slowly decays to a permanently inactivated state. This study further characterizes the properties of this "intermediate". Comparison of the secondary structure of active and inactivated tryptase by circular dicrohism (CD) demonstrated a relatively small change in the CD spectrum after inactivation. Most notable was the disappearance of a negative absorption peak centered at approximately 230 nm in active tryptase. When monitored as a function of time, absorbance at 230 nm decreased at a rate comparable to activity loss, suggesting that the change in the CD spectrum could be associated with formation of the reversible intermediate. The specificity of the re-activation process for heparin also was analyzed. About 50% maximal recovery of activity occurred at a heparin concentration appoximately equivalent to inactive tryptase, implying that binding was necessary for reactivation. Similar results were obtained with dextran sulfate, a highly sulfated polymer of glucose. Less recovery was achieved with glycosaminoglycans less sulfated than heparin and no reactivation was achieved with unsulfated dextran. These studies indicate that tryptase inactivation involves a relatively small conformational change, consistent with the formation of a reversible intermediate. Reactivation of this intermediate appears to be a non-specific process involving binding of negatively charged polymers by electrostatic interactions.

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IMMUNOGENETIC ANALYSES IN PATIENTS WITH SEVERE SKIN REACTIONS: CYTOKINE GENE POLYMORPHISM. Berthold Rzany, Gerald Messer[1], Stephanie Rämisch[1], Maja Mockenhaupt, Susan Baur, Judit Mueller, Ulrich Stocker, Jan Simon, Peter Kind[1], Erwin Schöpf, Department of Dermatology, Albert-Ludwigs-University of Freiburg; [1]Department of Dermatology, Ludwig-Maximilians-University of Munich, F.R.G.

Erythema exsudativum multiforme with mucosal involvement (EEMM), Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) belong to the most severe skin reactions in concern with partial or total epidermal necrosis and morbidity. Their occurance is often associated with administration of drugs and/or infectious diseases. The onset with fever, target lesions or exanthema followed by blisters and erosions in association with severe mucosal involvement is characteristic and points to the role of cellular activation, rapid mediators of inflammation, e.g. cytokines and molecules involved in mediating apoptosis. Tumor necrosis factor (TNF)-α and -ß, TNF receptors (TNFR p55, p75) and the fas-antigen/Apo-1 are such candidates. In order to study the epidemiological and pathophysiological bases, a registry was established with the aim to collect all cases of EEMM, SJS and TEN leading to hospitalization in the area of former West Germany and Berlin. Within this study, more than 1500 departments of dermatology, pediatrics, intensive care and burn units are contacted regularily. From 4/1/1990 to 4/31/1993, more than 527 patients have been interviewed.

We have isolated genomic DNA from PBMC of 48 patients, EEMM (12), SJS (18), TEN (15) and 179 random healthy individuals. Three cases could not be classified by the clinical definitions. PCR amplification of genomic regions of interest was performed. Using Nco I restriction, two alleles of TNF-ß can be detected within the human TNF-ß gene (TNFB*1 and TNFB*2). Previously the TNFB*1 allele has been linked to a higher production of TNF-ß upon stimulation of PBMC – *in vitro*. Phenotype (6%) and allele frequencies (0.25) of SJS and EEMM (8%, 0.29) are reduced for TNFB*1, when compared to the controls (11%, 0.33). In contrast, the allelic distribution of TNFB*1 in TEN (0.30) and the total collective of patients (N=48; 0.30) did not differ from the controls (0.33). Due to the small number of cases, only the analysis of a larger collective of severe skin reactions in this ongoing prospective study might prove a pathophysiological role of TNF-ß polymorphism. Currently, a polymorphism within the TNF-α promoter and search for structural differences in the regulatory regions of the lymphotoxin-ß gene are under investigation.

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EXPRESSION OF SIALYL LEWIS^x ON EPIDERMAL LANGERHANS CELLS IN THE HUMAN SKIN. Keiji Ohta, Mayumi Fujita, Yuji Horiguchi, Fukumi Furukawa and Sadao Imamura, Department of Dermatology, Kyoto University Faculty of Medicine, Kyoto, Japan

A carbohydrate antigen sialyl-Lewis^x(s-Le^x) is well known as a tumor-associated antigen. Recently, it has been demonstrated that the s-Le^x molecule is also present on leukocyte and serves in the process of inflammation as a ligand for endothelial-leukocyte adhesion molecule-1 which is expressed in the activated endothelial cells of blood vessel walls.

We examined the expression of s-Le^x in the human skin by immunohistochemical staining. Cryostat sections were stained with the avidin-biotin-peroxidase complex procedures. A few s-Le^x positive dendritic cells were observed in the epidermis of the human skin. To make clear whether these cells are Langerhans cells or not, we used anti-CD1a antibody and monoclonal Lag antibody that specifically reacts with Birbeck granules and related structures of human Langerhans cells. Double imunofluorescence studies revealed that s-Le^x positive dendritic cells in the epidermis were CD1a-positive and Lag-positive. These results suggest that s-Le^x epidermal dendritic cells are Langerhans cells and s-Le^x may be a new and useful marker of Langerhans cells.

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STUDY ON THE PATHOGENESIS OF ERYTHEMA INDURATUM. Qingsheng Mi, Ming Chen*, Hongdou Chen**. Dept.of Dermatology, Taishan Medical College, Taian, China. *The Second Military Medical University, Shanghai. **China Medical University, Shenyang, China.

The pathogenesis of EI were studied systematically in 21 patients with EI using direct immunofluorescence(DIF), ABC immunohistochemical method, enzyme linked immunosorbent assay (ELISA), RBC C3b receptor rosette and immune complexe rosette tests. The results were as follows: (1) DIF showed that three kinds of tissue-deposited immune complexes (TIC), IgA , IgG and IgM-, were deposited in the walls of blood vessels in 90% of EI and mainly IgM-TIC (85.7%). (2) Three CICs (IgA-,IgG-,IgM-) in sera examined using anti-C_3 ELISA were 85.7% positive and mainly IgG ,IgM in EI.There was a significant relativity between IgG-CIC and IgG TIC,IgM-CIC and IgM -TIC.The amount of CICs in sera were significantly reduced ($P<0.01$) after treatment. (3) The analysis of T cell subsets in the lesions of EI showed that mononuclear cells in the infiltrates were mainly T lymphocytes. (4) ABC immunohistochemistry of S100,Leu6 and HLA-DR in cryostat consecutive sections showed many dentritic and oval cells, which were positive for all of three antibodies at same time, were located in the lymphohistiocytic infiltrate of the deeper dermis and subcutis. From these results,we conclude that: (1) EI is a kind of immune complexe type vasculitis, and CIC play an important role in EI. (2)the so-called S100-positive dendritic cells found in the cutaneous inflamatory area of EI are LC, and LC are not only located in the epidermis and dermis, but also in the subcutis, which may present antigen to T cell and mediate type IV hypersensitivity in EI. (3) red blood cell immune function in EI is abnormal, which may be related to EI.

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NORMAL INDIVIDUALS HAVE ANTIBODIES TO HAIR FOLLICLE ANTIGENS WHICH ARE MODULATED DURING THE HAIR GROWTH CYCLE. Desmond J. Tobin and Jean-Claude Bystryn. The Ronald O. Perelman Dept. of Dermatology, NYU School of Medicine, New York, NY, USA.

We have previously shown that most normal individuals have autoantibodies to hair follicles. To further investigate the immunobiology of hair, we examined whether the expression of the antigens which are targeted by these antibodies is modulated during the hair growth cycle.

Anagen and telogen hair follicles were plucked from the scalp of a normal adult male. Anagen and telogen follicles were identified microscopically by the presence (anagen) or absence (telogen) of a well pigmented bulb and prominent outer root sheath, and collected separately. One hundred hair follicles of each type were extracted overnight with 0.5% NP-40, the supernatants run on SDS-8% PAGE, and tested for reactivity with sera of 7 normal individuals (diluted l/20) by Western immunoblotting. All individuals had antibodies to multiple hair follicle antigens. There were striking differences in the reactivity of these antibodies with anagen and telogen follicles. The antibodies reacted to antigens with MWs of approximately 120, 115, 110, 80 & 75 kDs which were strongly expressed in anagen hair follicles but were undetectable in telogen follicles. By contrast, the antibodies reacted strongly to a 55 kD antigen expressed by telogen, but not by anagen, follicles. Non-reduced extracts of telogen follicles also strongly expressed a broad antigen band in the 140-200 kd region which was not expressed in anagen follicles.

In summary, most normal individuals have antibodies to hair follicle antigens whose expression is modulated by the hair growth cycle. The expression of distinct autoantigens in the anagen and telogen phase of hair growth supports the hypothesis that immune factors may play a role in the regulation of hair growth and in certain hair diseases.

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DISTRIBUTION OF CD45RA+ AND CD45RO+ T LYMPHOCYTE SUBSETS IN SKIN LESIONS IN GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION. Hatsumi Fujii and Masaru Ohashi, Department of Dermatology, Nagoya University School of Medicine, Nagoya, Japan

Two monoclonal antibodies, anti-CD45RA and anti-CD45RO, reciprocally divide the CD4+ and CD8+ lymphocytes into CD4+CD45RA+, CD4+CD45RO+, CD8+CD45RA+ and CD8+CD45RO+ subsets. The CD4+CD45RA+, CD4+CD45RO+, CD8+CD45RA+ and CD8+CD45RO+ lymphocytes possess suppressor-inducer, helper-inducer, suppressor-effector and cytotoxic functions respectively. Distribution of CD45RA+ and CD45RO+ T lymphocyte subsets in skin lesions in graft-versus-host disease (GVHD) after allogeneic bone marrow transplantation (BMT) has not been known yet. We have characterized the infiltrated cells in skin lesions of 14 patients with GVHD after allogeneic BMT by immunohistochemistry using monoclonal antibodies directed against CD3, CD4, CD8, CD45RA and CD45RO.

In skin lesions in GVHD, a mojority of infiltrated cells were CD45RO+CD3+ T lymphocytes. In contrast, a small number of CD45RA+CD3+ T cell was observed. The predominant infiltrated lymphocytes within the epidermis were CD8+CD45RO+ cytotoxic T cells, and CD4+CD45RO+ helper-inducer or CD8+CD45RO+ cytotoxic T cells predominated in the dermis.

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COMPARISON OF WASTING SYNDROME IN [MRL lpr/lpr-MRL+/+] CHIMERA AND GRAFT VERSUS HOST DISEASE IN [B10.D2-BALB/c] CHIMERA AND AN ATTEMPT TO TRANSFER THE WASTING SYNDROME IN [MRL lpr/lpr-MRL+/+] CHIMERA TO MRL+/+ MICE. Michiko Aihara and Hiroshi Nakajima, Department of Dermatology, Yokohama City University School of Medicine, Yokohama, Japan

We compared wasting syndrome in [MRL lpr/lpr-MRL +/+] chimera with chronic GVHD in [B10.D2-BALB/c] chimera not only symptomatically but also histologically and studied whether only one portion of difference in minor histocompatibility can induce GVHD. BALB/c mice were lethally irradiated and given B10.D2 spleen cells and bone marrow cells. These mice are identical at the MHC and Mls but differ in the genetic background. These BALB/c recipients, [B10.D2-BALB/c] chimera, showed hair loss, weight loss and atrophy of lymph nodes and spleen from 5 weeks after transplantation as a result of chronic GVHD. MRL lpr/lpr mice carry the lpr gene and spontaneously develop generalized lymph node swelling and lupus-like autoimmune disease, while congenic MRL +/+ mice are 99.6% homozygous to MRL lpr/lpr mice, but lack the lpr gene. Wasting syndrome that developed the same symptoms as in [B10.D2-BALB/c] chimera was observed in [MRL lpr/lpr-MRL +/+] chimera mice from 14 weeks after cell transfer. Histologically, skin biopsy showed similar changes to human GVHD with HE staining, increase of Ia+ epidermal keratinocyte and decrease of Ia+ dendritic epidermal cells with immunoperoxidase staining in both chimera mice. Furthermore, we succeeded in transfer of wasting syndrome in [MRL lpr/lpr-MRL +/+] chimera to another MRL +/+ mice by transplantation of spleen cells from [MRL lpr/lpr-MRL +/+] chimera to lethally irradiated MRL +/+ mice.

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IDENTIFICATION OF ANTI-GOLGI ANTIBODY BY DOUBLE-STAINING METHOD WITH ANTIBODY TO β-COP. Hong-Shang Hong, Yu-Shung Lee, Heng-Leong Chan, Tseng-tong Kuo*, Departments of Dermatology and *Pathology, Chang Gung Memorial Hospital, Chang Gung Medical College, Taipei, Taiwan

Anti-Golgi Antibody (AGA) was rarely reported. During screening test of anti-nuclear antibody (ANA) for autoimmune disease patients by indirect immunofluorescence (IIF), AGA was detected as a speckled cytoplasmic staining pattern which partially surrounded the nucleus, just outside the nuclear membrane of HEp-2 cells. Furthermore, under immuno-electron microscopy (IEM), a strong immuno-histochemical staining was seen in the Golgi complex of the macrophages in the lymph node and in the area around the preacrosome of the spermatids in the testis.

Localization of AGA was further confirmed by double-staining of normal rat kidney cells with Anti-Golgi Antibody and antibody to β-COP. The result showed identical profile as the positive reaction at perinuclear regions.

Anti-Golgi Antibody was detected by indirect immunofluorescence and immuno-electron microscopy and confirmed by double-staining method with antibody to β-COP which is a component of non-clathrin-coated vesicles and recently known to be a good marker for Golgi complex.

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A QUANTITATIVE ANALYSIS OF NEUTROPHIL ADHESION IN BEHÇET'S DISEASE USING A NEW RHEOLOGICAL METHOD. Shigeruko Iijima, Fujiko Otsuka, Department of Dermatology, Institute of Clinical medicine, University of Tsukuba, Ibaraki, Japan

Behçet's disease is a recurrent inflammatory disorder with unknown etiologies. Since the inflammatory process is initiated by the adhesion of activated neutrophils to vascular endothels, such adhesive nature of neutrophils may be very important for Behçet's disease. In order to evaluate the presumption, we examined the adhesiveness of neutrophils using micropore filtration method, a newly-developed rheological method.

Peripheral blood was taken from 17 Behçet's disease patients and 17 healthy individuals. Each of neutrophil-rich blood (5,000 neutrophils/mm^3) was measured in terms of micropore transit time through a micropore filter with ϕ5 μm pores, which experimentally reflects the adhesiveness of neutrophils.

Micropore transit time was longer in whole blood suspension and neutrophil-rich suspension of Behçet's disease patients than in those of healthy individuals. When the patient's neutrophils were treated with their activating agents, they further showed longer transit time, stronger adhesiveness with statistical significance.

Our results indicate that the neutrophils of Behçet's disease patients are strongly adhesive to the micropore, which are furthermore reactive to neutrophil activating agents. This unique nature of neutrophils, strong adhesiveness and high response may contribute to a possible mechanism of recurrent and intractable oral and gential aphtous ulcers in Behçet's disease.

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EXPRESSION OF FAS ANTIGEN ON HUMAN KERATINOCYTES IN VIVO AND IN VITRO, AND INDUCTION OF APOPTOSIS. Koji Sayama, Shin Yonehara*, Satoshi Shiraishi and Yoshiharu Miki. Department of Dermatology, Ehime University School of Medicine, Ehime and *Pharmaceutical basic research laboratories, JT Inc., Yokohama, Japan

Fas Ag is a membrane protein inducing apoptosis, an important mechanism to maintain the homeostasis of the living tissues. The in vitro and in vivo expressions of Fas Ag on human keratinocytes were studied by immunohistochemistry and cell sorter. Apoptosis was determined by DNA fragmentation. Fas Ag and intercellular adhesion molecule-1 were co-expressed on the epidermis of various inflammatory dermatoses. Expression of Fas Ag on the cultured human keratinocytes was increased by the treatment with interferon-γ. Treatment of the cultured keratinocytes with anti-Fas antibody followed by interferon-γ resulted in 30 % cell death and DNA fragmentation. Apoptosis via Fas Ag may play an important role in the epidermal injury of various inflammatory dermatoses.

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THE PROGNOSTIC SIGNIFICANCE OF HLA ANTIGENE SYSTEMS IN PORPHYRIA CUTANEA TARDA (PCT). N.Kuznetsova, A.Chaschin I.Malov, Department of Dermatology, Medical Institute, Irkutsk, Russia.

The prognostic significance of HLA antigene system in different clinical forms of PCT was the aim of our investigation. 50 patients with PCT were examined (42 men and 8 women) at the age from 41 to 60 years. Among them 8 patients had only dermatological sings, I5 - neuro -dermatological, 2I - dermatological and visceral symptom and 6 mixed forms (clinical and laboratory tests of hepatic disturbance and neurological symptoms). The control group consisted of I84 Russian healthy people, aboriginals of the Easten Siberia. It was estimated a freguent occurrence of HLA AI9 (II,9%) and B40 (8,5%) in population of the Easten Siberia relatively with the Westen regions of Russia. The distribution of the most HLA antigene at the patients with PCT is egual to the freguent occurrence of the control groups. Nevertheless our investigations revealed some immunogenetic markers of PCT. At the subjects with HLA BI8 was warked the increased sensitivity to this disease(PP=3,9; p<0,00I). Besides PCT with HLA BI8 is associated with clinical and laboratory test of the hepatic disturbance (PP=I0,5; p<0,0I). These results suggest that the genetic state of patients determines not only the predisposition to manifestation of PCT but also determines the clinical forms of the disease.

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PLATELET ACTIVATING FACTOR (PAF) AND LYSO-PAF IN NORMAL AND INFLAMED SKIN Mary R Judge, RM Barr, AI Mallett, F Lawlor, A Kobza-Black, MW Greaves. St John's Institute of Dermatology, St. Thomas' Hospital, London, UK.

Platelet activating factor has been implicated in physiological, inflammatory and hypersensitivity processes and, injected intradermally in man, it elicits acute and delayed inflammatory reactions. Lyso-PAF, both the precursor and metabolite of PAF, is thought to be biologically inactive. Traditionally, PAF has been detected by bioassay. We developed a sensitive gas chromatography-mass spectrometry assay for PAF and lyso-PAF[1].

Mean (+/-sem) PAF and lyso-PAF levels in suction blister fluid of 8 healthy subjects were 1.86 +/- 0.9 and 160 +/- 21nM respectively. Time course studies showed a constant rate of PAF release over 2 hrs. Topical clobetasol propionate 0.05%, for 72hrs, failed to suppress PAF release but significantly reduced lyso-PAF concentrations in blister fluid in 8 subjects.

Thirteen patients with untreated chronic plaque psoriasis showed no significant difference in PAF levels between lesional and non-lesional skin exudates but had significantly more lyso-PAF in lesional skin (9.5 +/-1.9 compared to 3.14 +/-0.43 pmole/sample in non-lesional skin). A similar pattern was seen in seven patients with nickel allergic dermatitis. Blister fluid of 6 patients with delayed pressure urticaria yielded similar amounts of PAF and lyso-PAF from control skin and from lesions at 0 and 6 hrs post pressure challenge. Seven atopic patients, challenged with house dust mite on abrasions, showed no significant variation in PAF and lyso-PAF levels at 0.5, 5.5 and 6 hrs.

Lyso-PAF predominated over PAF in normal skin and its release was inhibited by a potent steroid. It occurred in significantly higher amounts in lesions of psoriasis and contact allergic dermatitis. Surprisingly, PAF levels were not affected by steroid and remained low in the inflammatory and hypersensitivity dermatoses tested. PAF may be a significant mediator in inflamed skin but these results suggest that it is either degraded rapidly or remains largely cell-associated.

1. Mallett A, Barr R et al. Release of platelet activating factor from human skin. J Invest Dermatol 1991, 96:p1007.

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DETACHMENT OF CULTURED NORMAL HUMAN KERATINOCYTES BY CONTACT WITH TNF α-STIMULATED NEUTROPHILS IN THE PRESENCE OF PAF. Hiroshi Katayama[1], Tadashi Hase[2], Hideo Yaoita[1], [1]Department of Dermatology, Jichi Medical School, Tochigi, Japan and [2]Department of Biology, Kao Biological Science Laboratory, Tochigi, Japan

This study was aimed to see and analyse biological phenomena that might take place in cell-cell contact between neutrophils and keratinocytes. Normal human neutrophils were plated on cultured normal human keratinocytes (approximately 8:1 in number) and co-cultured for 16-24 h in serum-free media. As a result, keratinocytes were conspicuously detached under a special condition. Detachment rate was obtained by counting the number of undetached keratinocytes. Addition of unstimulated neutrophils hardly evoked the detachment. Addition of TNFα-stimulated neutrophils alone or unstimulated neutrophils together with 10^{-5}M PAF caused only partial detachment. However, TNFα-stimulated neutrophils added together with 10^{-5}M PAF (referred to as TNF-PAF line) caused conspicuous detachment (detachment rate, 77.1±13.0% in seven separate experiments). Inhibiting the direct contact of neutrophils with keratinocytes in TNF-PAF line with membrane filter, detachment decreased remarkably. Addition of anti-CD18 to TNF-PAF line without membrane filter inhibited the detachment by only 12.6%. α_1-proteinase inhibitor added to TNF-PAF line inhibited the detachment nearly completely and a synthetic elastase specific inhibitor, ONO-5046, completely. α_1-antichymotrypsin inhibited it by 39.0%. These results indicate that TNF α-stimulated neutrophils were raised to a higher activation level by contact with keratinocytes in the presence of PAF and secreted elastase in a sufficient amount to elicit conspicuous detachment of keratinocytes. This study may be a model of subepidermal blister formation in the last stage of several bullous diseases with neutrophil infiltration such as dermatitis herpetiformis.

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THE IMMUNOPATHOLOGY OF IDIOPATHIC AND PRESSURE INDUCED URTICARIA.

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We have examined the inflammatory cell infiltrate in chronic idiopathic and delayed pressure urticaria (DPU) in relation to adhesion molecule expression on vascular endothelium. Three biopsies were taken from each of 13 patients with DPU, from a combination of unchallenged and/or pressure challenged skin at 0, 2, 6, 24, 48 and 120 hours after weighted rods (3.5 kg) were applied to the thighs for 20 minutes. Three biopsies were obtained from each of 4 normal controls, both before and at 6, 24 and 48 hours after an identical pressure challenge. Biopsies were also taken from spontaneous weals in 4 patients with chronic idiopathic urticaria but not DPU. Chloracetate esterase was used to stain mast cells and an immunohistochemical technique to demonstrate the inflammatory cell subsets and the vascular adhesion molecules; E Selectin, Intercellular Adhesion Molecule 1 (ICAM-1) and Vascular Cell Adhesion Molecule 1 (VCAM-1).

E Selectin was moderately to markedly upregulated in DPU patients at 6 and 24 hours after pressure and most patients had increased expression of VCAM 1 on perivascular cells at these times. There was no clear trend in expression of ICAM-1. There was a significant rise in the median count per high power field of neutrophils (neutrophil elastase) at 6 hours after pressure with a further rise at 24 hours ($p<0.05$, Mann Whitney U). The median cell counts of eosinophils (eosinophil cationic protein) and monocyte/macrophages (EBM 11) also increased significantly at 24 hours. In the DPU patients, the median mast cell count at 6 hours after pressure had decreased significantly and was similar to that in idiopathic urticaria, suggesting that mast cell degranulation may occur in both disorders. In chronic idiopathic urticaria, the cell infiltrate was similar in composition but intermediate in intensity to that seen in DPU at 6 and 24 hours. Biopsies from 4 normal controls subjected to a pressure challenge showed no detectable changes in either adhesion molecule expression or the cell infiltrate.

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IMMUNOPHENOTYPING OF ISOLATED HUMAN SKIN MAST CELLS.

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Methods for the isolation and purification of mast cells from human skin by using enzymatic digestion have permitted us to analyze the biochemical and morphological characteristics of these cells and to make more intensive comparison with mast cells from other anatomical sites. In this study, we used immunohistochemical methods to determine whether isolated adult human skin mast cells (SMC) express surface antigens, such as adhesion molecules of the integrin, a variety of leukocyte antigens, and newly identified high affinity IgE receptor. By enzymatic digestion of the adult skin and centrifugation on discontinuous percoll density gradient, approximately 1×10^4 mast cells were recovered from one gram (wet weight) tissue. 50%-75% of the cells in cytocentrifugation speciemens were mast cells, assessed with alcian blue staining. Using APAAP immunohistochemical staining, we examined the expression of cell surface antigens. Consistent with previous reports examining surface markers on human lung or uterine mast cells, SMC were stained HLA class I, IgE, CD29, CD43, CD45, CD49d, CD49e, CD61 and CD68, however CD11b, CD23, CD49b were not stained. In contrast to previous reports on other mast cells, SMC expressed CD11a and CD49f weakly, but CD11c, CD18, CD54 and c-kit were not detected.
Additional surface molecules present on SMC included high affinity IgE receptor which was detected by two different monoclonal antibodies. We also investigated the phenotype of mast cells in solitary mastocytosis, showing strongly positive staining of HLA-DR. In this presentation, we report that SMC possess unique phenotypic characteristics, probably responsible for the immunological reaction in skin.

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EFFECT OF DILTIAZEM ON MURINE EPIDERMAL LANGERHANS CELLS.

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Systemic diltiazem was shown to diminish the density of Ia-positive epidermal Langerhans cells (ELC). It was unclear, however, whether this effect reflects ELC death or only the loss of surface expression of class II MHC antigens. In this study, we examined whether the proportion of Ia-negative ELC increase in the mice pretreated systemically with diltiazem. We also studied the effect of topical diltiazem on the density of murine ELC.

Epidermal sheets was separated from the dorsal ear skin of the BALB/c mice pretreated systemically or topically with diltiazem, followed by immunohistochemical staining for mouse I-A^d and the density of Ia-positive ELC was calculated. The half of the epidermal sheets were processed for immunoelectron microscopy for mouse I-A^d and then the number of Ia-positive and -negative ELC detected by the existence of Birbeck granules was counted.

The density of Ia-positive ELC were significantly decreased after treatment with both systemic and topical diltiazem. Some of them showed round cell body and short dendritic processes. In pretreated mice, as well as in control, Ia-negative ELC could be hardly recognized by immunoelectron microscopy.

Topical, as well as systemic, diltiazem has the effect to diminish the density of Ia-positive ELC and it reflects not only the loss of surface expression of class II MHC antigens but ELC death.

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THROMBOMODULIN AS A MARKER FOR MONOCYTIC/HISTIOCYTIC INFILTRATION IN CHRONIC GRANULOMATOUS INFLAMMATION.

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Thrombomodulin (TM) is a surface glycoprotein that forms a stoichiometric complex with thrombin thereby serving as a natural anticoagulant on endothelium of arteries, veins, capillaries, and lymphatics. Recently McCachren et al. (1991) reported TM expression by human blood monocytes and by human synovial tissue lining macrophages. Further Conway et al. (1992) reported human neutrophils synthesize TM. We here studied immunohistochemical expression of TM-positive inflammatory exudate cells in the granulomatous inflammation. Tissue sections from lupus miliaris disseminatus faciei (n=7), sarcoidosis (n=4), sporotrichosis (n=4), and chromomycosis (n=1) were collected. Anti-human recombinant TM polyclonal rabbit antiserum was used with avidin-biotin peroxidase complex immunohistochemistry technique. Immunoreactivity to S-100, vWF, UEA-1, lysozyme, and CD68 was also investigated. Results clearly showed that monocytic histiocytes infiltrating into the tissue demonstrated TM-positive reaction. They are not capillary-forming but round or cuboidal cells with dendrites. These TM-positive cells were formed perivascularly and surrounding the granulomas but not observed in the center of organized granulomas where epithelioid cells and multinucleated giant cells were developed. Furthermore double immunostaining technique revealed that TM-positive cells belong to separate group distinct from lysozyme-positive and CD68-positive histiocytes. In conclusion TM is a good immunomarker for monocytic histiocytic in the early infiltrating and pre-granuloma forming stage, while TM is lost as mononuclear phagocytes undergo further maturation in the granulomas.

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PUVA CAN SUPPRESS THE EXPRESSION OF CELL ADHESION MOLECULES AND SURFACE ANTIGENS OF PERIPHERAL BLOOD MONONUCLEAR CELLS.

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Accumulation of an activated T lymphocyte is the primary stage of the inflammatory skin diseases including psoriasis vulgaris (PV) and the cell adhesion molecules play an important role in this reaction. To know the therapeutic mechanism of PUVA on PV, the effects of PUVA on the expression of cell adhesion molecules and surface antigens of peripheral blood mononuclear cells (PBMC) were investigated. PBMC obtained from healthy volunteeres were activated by Con A and were irradiated with UVA light in the presence of 8-MOP. The expression of LFA-1, VLA-4, CD4, CD8 and CD25 were stained by monoclonal antibodies and analyzed by FACScan. PUVA treated Con A blasts showed the suppressed expression of LFA-1, VLA-4, CD4, CD8 and CD25. PBMC obtained from PV patients 24 hours after oral PUVA therapy showed significant decreased expression of CD25. These results suggest that the effects of PUVA on PV induces via the suppression of the expression of cell adhesion molecules and surface antigens of T lymphocytes.

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DIAGNOSTIC POTENTIALS FOR ONE AND TWO-STEP TARGETING STRATEGIES IN LANGERHANS CELL HISTIOCYTOSIS.

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In this study we aim to tailor the development of two-step targeting in order to: 1) Increase specificity of 2nd antibody, 2) Provide subtle amplification of signal, 3) Increase scope of system by developing a universal agent.

Such systems were developed with respect to Langerhans cell histiocytosis (LCH) via the cell surface antigen Placental Alkaline Phosphatase (PLAP). Indirect immunohistochemistry of BeWo and HEp2 cell lines, term placental tissue and frozen and paraffin embedded LCH tissue, all expressing PLAP were used in the analysis of whole Ab, hapten derivitised Ab - scFv two-step system, scFv and scFv effector molecule diagnostic targeting potential.

We demonstrate the effective manipulation of MoAb H17E2 (antigen - PLAP) both at the chemical and recombinant level generation of recombinant scFv's, recombinant scFv-effector molecules and hapten derivitised two-step targeting approaches concluding that: a) Hapten derivitisation allows for increased specificity and signal amplification, b) Recombinant scFv-effector molecules offer signal amplification, c) scFv's offer as a diagnostic tool with potential in paraffin embedded tissue.

These systems may prove useful in the diagnosis of LCH in paraffin embedded tissue, and may open the way for generation of similar systems for other disorders.

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SECRETORY COMPONENT AS AN INTRINSIC INHIBITOR FOR GAMMA-INTERFERON OF KERATINOCYTES. Yoshimichi Nihei, Kohji Maruyama, Jan-Zhong Zhang and Fumio Kaneko, Department of Dermatology, Fukushima Medical College, Fukushima, Japan

Recent reports have revealed that many kinds of cytokines are contributing to the pathogenesis of psoriasis vulgaris, especially gamma-interferon (IFN-γ) is important for the development of psoriatic lesions. On the other hand, we have already reported that the presence of secretory component (SC) and secretory IgA (sIgA) in the lesions of psoriasis and some other dermatoses. To make it clear the roles of SC and sIgA in inflammatory dermatoses, we examined SC expression on cultured human keratinocytes in stimulation of IFN-γ, and the inhibitory activities of SC against the effects of IFN-γ on keratinocytes using flow cytometry.

As the results, SC was induced by IFN-γ on keratinocytes, but the responses were vary in each strain. Free SC (FSC) was able to suppress ICAM-1 and HLA-DR expression on keratinocytes in stimulation of IFN-γ. The inhibitory activity of FSC was related to phospholipase A2 inhibition because of *p*-bromophenasyl bromide showing similar inhibition. Lipocortin would not be implicated in this inhibition, because the effect of FSC was much stronger than that of dexamethasone.

These results may suggest that SC, which is inducible on keratinocytes by IFN-γ, works as an intrinsic inhibitor for IFN-γ. Such inhibitory activity for IFN-γ may be of important for the pathogenesis of psoriasis vulgaris.

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APPLICATION OF GAS CHROMATOGRAPHY TO PROVIDE AN ESTIMATION OF LIPASE ACTIVITY OF PROPIONIBACTERIUM ACNES

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We used gas chromatograpy to estimate the lipase activity in clinical strains of Propionibacterium acnes. The lipase activities of P. acnes strains were estimated by the amounts of butyric acid which appeared in Peptone-Yeast extract Glucose medium added with tributyrin after incubation at 37°C for 72 hrs. The optimal concentration of tributyrin in the medium was 0.017meq/ml. Tributyrin in the medium did not inhibit the lipase activities of P. acnes strains. This new method might be useful for measurement of the lipase activity of P. acnes and the anti-lipase activity of Kampoh drugs (Combination of Japanese-Chinese medicines), crude drugs, and antibiotics on the organism.

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THE CORRELATION OF KAMPOH FORMULATIONS AND THEIR INGREDIENTS ON ANTI-BACTERIAL ACTIVITIES OF PROPIONIBACTERIUM ACNES. Shuichi Higaki, Yoshinori Hasegawa and Masaaki Morohashi, Departmentof Dermatology, Toyama Medical and Pharmaceutical University, Toyama, Japan

P. acnes strains were tested for the correlation of their susceptibility to the Kampoh formulations, Seijo-bofu-to (SBT), Keigai-rengyo-to (KRT) and their 18 ingredients (Kampoh crude drugs). Generally, SBT and KRT are low susceptible to P. acnes. On the other hand, two ingredients, Coptidis Rhizoma (CR) and Phellodendri Cortex (PC) are high susceptible to P. acnes. In 7 out of 17 Kampoh crude drugs in KRT and 5 out of 11 in SBT, the value of MIC_{80} of the high sensitive P. acnes group are lower than that of the low sensitive P. acnes groups. The remarkable propotion of MIC of P. acnes between KRT and SBT, KRT and CR, KRT and PC, SBT and CR was not observed.

From these results, anti-bacterial activities to P. acnes of Kampoh crude drugs by interaction was suggested. Furthermore, Kampoh formulations, composed of CR, PC and less numbers of other Kampoh crude drugs, might be showed high sensitive to P. acnes, such as Oren-gedoku-to, and proved clinical effectiveness to acne patients. To prove this basically, it must be assayed the absorbtion, secrection and concentration of them.

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EFFECT OF ROXITHROMYCIN ON NEUTROPHIL REACTIVE OXYGEN SPECIES : ANOTHER POSSIBLE MECHANISM OF ACTION IN ACNE. Hirohiko Akamatsu, Setsuko Nishijima, Hiroko Sasaki, Yukie Niwa and Yasuo Asada. Department of Dermatology, Kansai Medical University, Osaka, Japan.

Roxithromycin is a semi-synthetic macrolide antibiotic. On the basis of recent reports that roxithromycin is an effective medicine for acne and that tetracyclines are effective in acne by directly acting as antioxidant on infiltrated neutrophils, we investigated if roxithromycin is also capable of reducing the generation of reactive oxygen species (ROS) using human neutrophils and a cell-free, xanthine-xanthine oxidase system. The species investigated are superoxide radical anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH•). The formation of O_2^- was determined by measuring ferricytochrome c reduction induced by O_2^- produced from neutrophils stimulated with opsonized zymosan. H_2O_2 generation was measured by quantifying the weakening of fluorescence intensity of scopoletin due to its peroxidase-mediated oxidation by H_2O_2. OH• was quantitated by taking the amount of ethylene gas formed from α-keto-methiolbutyric acid plus the neutrophil-generated OH•. Roxithromycin significantly decreased the generation of O_2^-, H_2O_2, and OH• by neutrophils, whereas it did not markedly inhibit the ROS levels generated in a cell-free, xanthine-xanthine oxidase system. The present study seems to suggest that roxithromycin besides its well known effect on microbes has an additional property in acne via the antiinflammatory route by reducing oxidative tissue injury.

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IMMUNOHISTOCHEMICAL STUDY ON PROLIFERATING CELLS IN ALOPECIA USING PROLIFERATING CELL NUCLEAR ANTIGEN. Yoon Whoa Cho, Hak Kyu Lee, Kye Yong Song*, Byung In Ro Departments of Dermatology & Pathology, College of Medicine, Chung Ang University, Seoul, Korea

The number of proliferating cell populations in the hair follicles is closely related to the regulation of hair growth. And destruction of hair germ cells and loss of proliferating cells has been suggested to be of major pathogenetic significance in alopecia areata. The purposes of this study is to compare the growth fractions in proliferating cells of hair follicles in normal with in alopecia. An immunohistochemical study was done to detect the proliferating cells in hair follicles using a monoclonal antibody against proliferating cell nuclear antigen(PCNA) in the scalp biopsy of alopecia patient on the paraffin embedded sections. Significant increase of the labelling indeces of PCNA were observed in the hair follicles of alopecia compared with normal condition. But these lesions could not find significant differences of labelling index in the clinicla thpes of alopeica, responses to treatment, and systemic immunologic state. With above results is confirmed that significant decreases of proliferating cells in the hair follicle could explain the part of the pathogenetic mechanism in alopecia, but it's direct cause which inhibit the proliferatiion of cells is not clear.

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COMPARATIVE ANALYSIS OF EXTRACELLULAR MATRIX SYNTHESIS BETWEEN COBBLESTONE SHAPED AND TUBE-FORMED VASCULAR ENDOTHELIAL CLONES, F-2 AND F-2C. Chiung-Shan Chen, Ken-Ichi Toda, and Sadao Imamura. Department of Dermatology, Kyoto University Faulty of Medicine, Kyoto, Japan

Angiogenesis process (AP) plays important role in many biological phenomena of health and diseases. Although AP is closely related to the activation of vascular endothelial cells (EC), it is not well known how this morphological and/or functional modulation of EC during AP is related to cytokines or extracellar matrix (ECM). We recently have established two murine EC clones, F-2 and F-2C. F-2 grow well in FCS supplemented DMEM media with cobblestone appearence, whereas F-2C grow in chemically defined media to form 3 dimentional structures of tubulogenesis. Those obervations suggest that F-2 and F-2C serve as useful models for studying proliferation and differentiation of EC in AP. In this study, we did the experiments to learn more about both clones, especially focusing to how these morphological differences between the both clones were related to the ECM production including fibronectin (FN), laminin (LM) and type IV collagen (COL IV). Immunohistochemical studies showed that F-2C secreted subcellular matrices on the plastic more strongly reacted to anti-,LM or COL IV antibodies than F-2. Western blot analysis revealed that the conditioned media by F-2C contained more amount of immunoreactive LM, FN and COL IV than that of F-2, and the cell lysates of F-2C contained more LM and FN than those of F-2, whereas the cell lysates of F-2 contained more COL IV than those of F-2C. These results indicate that (1) ECM production is differently regulated between F-2 and F-2C and (2) higher amount of basement membrane ECM (LM and COL IV) production in the medium and their extracellular reorganization to form basement membrane structures may be relevant to the histodifferentiation of F-2C.

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A FLUOROMETRIC RAPID MICROASSAY FOR THE ASSESSMENT OF FIBROBLASTS PROLIFERATION IN VITRO. Yoko Akai, Hirohiko Akamatsu and Yasuo Asada. Department of Dermatology, Kansai Medical University, Osaka, Japan.

It has been well accepted that counting of cell numbers and determination of [^{3}H] thymidine incorporation into cellular DNA are the methods used for the assessment of fibroblasts proliferation in vitro. However these methods have some problems, such as time-consuming. Recently a fluorometric rapid microassay for the determination of proliferation of keratinocytes, sebocytes and melanocytes in vitro has been reported. This assay is based on the hydrolysis of the fluorogenic substrate 4-methylumbelliferyl heptanoate (MUH), by cell esterases. In the present study, we describe a simple, fast and reliable assay using MUH for the determination of proliferation of normal human fibroblasts and 3T3 fibroblasts in vitro. Both of these cells were grown at several densities in 96-well culture plates and were incubated with MUH after 4, 6, and 8 days. The generated fluorescence showed a strong correlation with the cell number at various growth phases. Our results seem to suggest that this fluorometric rapid microassay with MUH as substrate is a simple, rapid and reproducible method for the assessment of fibroblast proliferation in vitro.

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FIBROBLAST-COLLAGEN INTERACTION: ITS ROLE FOR BIOSYNTHETIC FUNCTIONS OF FIBROBLASTS. C.Mauch, B.Eckes, E.Kozlowska, E. Klein and T.Krieg, Departments of Dermatology, Universities of Cologne and Würzburg, Germany

Connective tissue biosynthesis and degradation is strictly controlled under physiological conditions. Alterations of the equilibrium occur during wound healing and tissue regeneration, but also in fibrosis, tumor invasion and metastasis. It is mainly regulated by two mechanisms, the release of cytokines and the contact of cells with the surrounding ECM. We investigated therefore the influence of defined matrix components on synthetic and degradative potential of human skin fibroblasts. Cells were seeded into 3D-collagen gels. They interact with these collagen fibrils via specific membrane receptors of the integrin family and contract the loose collagenous network to a dense tissue. This process was inhibited by specific antibodies directed against $\alpha 2\beta 1$ integrins. Furthermore production of these integrins was induced during contraction. Compared to monolayers in contracted collagen gels fibroblasts displayed an altered morphology associated with changes of the cytoskeleton organization and a reduced proliferation. Analysis of connective tissue metabolism revealed a strong down regulation of collagens I and III by decreasing transcription and stability of the specific mRNAs. Synthesis of other matrix proteins including fibronectin and collagen VI was not affected. Synthesis of degrading enzymes of the metalloproteinase family was also found to be altered when cells were grown in contact to collagen. Both, the type I collagenase and the 72 kDa gelatinase were induced in synthesis and activation of the latent proenzymes. This indicates that the interaction of cells with the surrounding matrix has a major impact on the regulation of cellular functions and can contribute to the release of proteolytic activity during tissue remodelling and the invasive growth of tumors.

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IMMUNOHISTOCHEMICAL LOCALIZATION AND GENE EXPRESSION OF HUMAN LYSYL OXIDASE IN THE SKIN. Noriko Yashiro, Hiroyo Fushida, Miyako Chanoki, Hiromi Kobayashi, Tomoyuki Hisa, Shoji Taniguchi, Masamitsu Ishii, Toshio Hamada and Akira Ooshima*, Department of Dermatology, Osaka City University Medical School, Osaka, Japan, *Department of Pathology, Wakayama Medical College, Wakayama, Japan

Lysyl oxidase initiates the cross-linking of collagen and elastin by catalyzing the formation of the lysyne-derived aldehyde. The activity of lysyl oxidase in fibrotic conditions or inherited connective tissue diseases has been reported. However, little is known about the localization of it in human skin. We investigated the localization of lysyl oxidase in normal human skin and cultured human keratinocyte using indirect immunofluorescence method with monoclonal anti-human lysyl oxidase antibody. Fine filamentous or granular positive immunoreactions were noted on or among collagen or elastic fibers. Intracellular distribution of basal cells, endothelial cells and sweat glands also observed as well as that of fibroblasts. The cultured human keratinocytes exhibited a immunoreaction with a filamentous cytoskeletal protein-like structure. To rule out another possibility that certain proteins share haptens, total human keratinocytes RNAs were subjected to Northern analysis. We detected the expression of lysyl oxidase gene in human keratinocyte. The results suggest that lysyl oxidase would have other functions besides initiation of cross-linking in collagen and elastin.

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DETECTION OF LYSYL OXIDASE GENE EXPRESSION IN RAT SKIN DURING WOUND HEALING. Hiroyo Fushida[1], Michio Fukuda[1], Naoki Maekawa[1], Noriko Yashiro[1], Hiromi Kobayashi[1], Miyako Chanoki[1], Masamitsu Ishii[1], Toshio Hamada[1] and Syuzo Otani[2], Departments of [1]Dermatology and [2]Biochemistry, Osaka City University Medical School, Osaka, Japan, Chiyo Shiota and Akira Ooshima Department of Pathology, Wakayama Medical College, Wakayama, Japan

Lysyl oxidase initiates the cross linking of collagen and elastin by catalyzing the formation of the lysine-derived aldehyde, and plays an essential role for maturation of collagen and therefore for wound healing. Although the activity of this enzyme has been examined in various disorders, the biologic significance and the function in tissues are still unknown. We detected lysyl oxidase gene expression using Northern blot hybridization and in situ hybridization method in rat skin during wound healing. Moreover, its localization was investigated by the immunofluorescence method. Lysyl oxidase mRNA level reached a peak at 4 day and returned to the control level at 7 day after injury. In situ hybridization showed grains corresponding to its mRNA in fibroblasts around the granulomatous tissue. It is supposed that these results are fundamentally important and useful for the better understanding of collagen metabolism in vivo, and further elucidation of fibrotic disorders.

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IMMUNOLOCALIZATIONS OF HUMAN GELATINASE (TYPE IV COLLAGENASE, MMP-9) AND TIMP (TISSUE INHIBITOR OF METALLOPROTEINASES) ON THE SKIN. Takashi Kobayashi, Yutaka Nagai and Takeji Nishikawa, Department of Dermatology, Keio University School of Medicine, Tokyo Japan and Department of Tissue Physiology, Medical Research Institute, Tokyo Medical and Dental University, Tokyo Japan

For the tissue remodeling in both normal and inflammatory states, MMPs (matrix metalloproteinases) are considered to play important roles on the metabolism of tissue components which consists various kinds of organs. Especially, gelatinases (MMP-2 and MMP-9), which are also called as type IV collagenases, have been indicated to serve as critical enzymes which cleave the structural components of basement membrane zone. And that TIMP is also known as an important molecule because of its inhibitory effects on gelatinases. On these grounds, we have raised monoclonal antibodies against both one kind of gelatinase (MMP-9) derived from human polymorphonuclear leukocytes and human recombinant TIMP. By using immunohistochemical method, we investigated the immunolocalizations of these two kinds of molecules in the skin for each some cases of epidermal tumors including senile keratosis (SKs) and squamous cell carcinoma (SCCs). As a result, in the normal site remote from tumors, MMP-9 showed positive staining pattern in keratinocytes without basal cell layer in contrast to thorough positivity of TIMP in the epidermis. As with SKs, however, MMP-9 was positively stained even in basal cell layer. We also observed changes of staining pattern showing some destained part in the tumor cells of SCCs with TIMP. In conclusion, these results suggested us the possibility that the difference in the presence or absence of both MMP-9 and TIMP is closely related to the changes of benign tissue into malignant transformation.

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ADHESION OF EPIDERMAL LANGERHANS CELLS TO VARIOUS EXTRACELLULAR MATRIX PROTEINS MIGHT INFLUENCE THEIR MIGRATION BEHAVIOR. Marie-Jeanne Staquet, Yasunobu Kobayashi, Colette Dezutter-Dambuyant, Daniel Schmitt, Dermatology Research Unit, INSERM U346, CNRS SDI 6199, E. Herriot Hospital, Lyon, France

It is clear that epidermal Langerhans cells (LC) are capable of movement and of leaving the epidermis. Cell motility depends on the ability of cells to identify, attach and change shape in response to specific extracellular matrix (ECM) components. On the way to regional lymph nodes, while passing through the basement membrane (BM), LC get in contact with laminin (LM) and type IV collagen (Coll) at first and then with fibronectin (FN) and type I collagen in connective tissue of dermis. The present study was undertaken to learn more about the adhesive interactions between human LC and these ECM proteins. For this purpose, using highly enriched LC suspensions (70-90% LC), double adhesion assays were developed for the evaluation of the ability of LC to successively bind to two different substrates. If binding assays were first performed on LM-coated plates, the recovered attached LC were then plated on FN-, type IV- and type I Coll-coated dishes. Each of the four ECM proteins were thus tested in pairs to analyse the successive binding capacities of LC.

Our results show that i) the LC which first attach to LM are then able to attach to type IV Coll and vice versa, ii) LC which adhere to LM or type IV Coll are then able to attach to FN and to type I Coll, iii) LC which first adhere to FN or type I Coll preserve their ability to normally attach to type IV Coll, iiii) in contrast, the binding capacity to LM of LC which first adhere to FN or type I Coll is reduced by 50%.

These data indicate that following contact to basement membrane components, epidermal LC are able to successively attach to ECM proteins present in the dermis whereas LC, once attached to dermis ECM proteins, decrease their binding capacity to BM, especially to laminin. Interactions with the ECM environment seem to play a crucial role in the directed migration of epidermal LC from the skin to proximal lymph nodes.

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THE EFFECT OF PLATELET-DERIVED GROWTH FACTOR UPON CELLULAR INFILTRATION AND VASCULARISATION OF A CUTANEOUS COLLAGENOUS IMPLANT IN THE RAT.
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The use of collagenous implants offers one potential treatment of full thickness skin lesions, although results may not be ideal. We have investigated the effect of the recombinant BB homodimer of human platelet-derived growth factor (PDGF) upon wound healing following the implantation into full thickness excision wounds in the dorsal skin of rats, of a trial bovine atelocollagen sponge (collagen I:III, ca. 9:1) consisting of both fibrous and denatured collagen (9:1), stabilised by dehydrothermal crosslinking, and with an outer silicone layer. Before implantation, sponges were soaked in saline alone, or in saline containing PDGF, in amount such that the entire volume of liquid was absorbed.

Histological examination of excised sponges at 14 and 21 days post-implantation, in dose-response studies utilising between 0-4μg PDGF per wound, showed an enhanced infiltration of host cells, mostly fibroblasts, and enhanced capillary formation, under the influence of PDGF, the greatest response being observed with 4μg of this cytokine. The proportion of inflammatory cells invading the sponge was markedly less with PDGF, although a mild eosinophilia was apparent.

Despite the enhanced vascularisation, bromodeoxyuridine staining demonstrated that the process still did not occur sufficiently rapidly to allow the survival of epithelial cells in split-thickness skin grafts superimposed upon sponges lacking an outer silicone layer at the time of their implantation.

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PCR-BASED DETECTION OF TWO POLYMORPHISMS IN THE TYPE VII COLLAGEN GENE AND ITS APPLICATION FOR THE DNA LINKAGE STUDY ON THE FAMILY WITH DOMINANT DYSTROPHIC EPIDERMOLYSIS BULLOSA (DDEB).
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Type VII collagen is the major collagenous component of the anchoring fibrils. Recent study suggested there are two intragenic polymorphisms in the human type VII collagen gene. In this study, we determined the alleleic frequencies of PvuII and AluI polymorphism in the Japanese population and attempted to establish the genetic linkage between DDEB and type VII collagen gene. Genomic DNA was isolated from 40 unrelated healthy individuals and PCR was performed. The PvuII and AluI allelic frequencies are 0.45 and 0.36, respectively. Next,we examined these two polymorphisms in a Japanese family with DDEB. In this family, the affected individuals had blisters and erosions with onset shortly after birth. Electron microscopic observation showed the separation just beneath the basal lamina and the reduced number of anchoring fibrils. PvuII and AluI RFLP were analyzed in 9 affected and 13 unaffected individuals. Two-point linkage analysis revealed co-segregation of PvuII and AluI polymorphisms in this pedigree ($Z=2.15$; $\theta=0.00$). These data suggest that the type VII collagen is the candidate gene for this Japanese family with DDEB.

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FOCAL EXPRESSION OF TYPE VII COLLAGEN BENEATH HEMIDESMOSOMES IN DYSTROPHIC EPIDERMOLYSIS BULLOSA AND DURING WOUND HEALING.
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Type VII collagen (VIIc) is the major component of anchoring fibrils at the dermal-epidermal junction and is synthesized by keratinocytes. Synthesis of type VIIc may be deficient or defective in dystrophic epidermolysis bullosa (DEB). In contrast, synthesis of type VIIc may be increased during wound healing in normal human skin. To investigate the dynamics of type VIIc synthesis and expression at the protein level, we have performed a pre-embedding immunogold electron microscopy study using an antibody that labels part of the NC-1 domain of type VIIc. Intact skin samples from 4 patients with dominant DEB, 4 patients with localized recessive DEB, and 4 patients with severe generalized recessive DEB were studied. In addition, biopsies from normal skin and 10-day-old wounds were assessed in 2 control subjects. In the DEB samples, type VIIc labelling was seen on a range of fibrillar structures beneath the lamina densa showing variable structural resemblance to normal control immunogold-labelled anchoring fibrils. Notably, the immunolabelling was maximal immediately subjacent to hemidesmosomes. In milder DEB phenotypes, occasional intracellular type VIIc labelling was present within basal keratinocyte endoplasmic reticulum or actually overlying hemidesmosomes. In the normal wound healing samples, some of the immunolabelled anchoring fibrils had a wisp-like appearance, similar to those seen in non-wounded DEB skin. The immunogold labelling was again maximal beneath hemidesmosomes. This study raises the possibility of hemidesmosomes having a role in the secretion of type VIIc from keratinocytes into the extracellular space in both pathological states as seen in DEB and in normal biological responses such as wound healing.

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REGULATION OF TOPOISOMERASE I GENE EXPRESSION IN FIBROBLASTS FROM PATIENTS WITH SYSTEMIC SCLEROSIS
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DNA topoisomerases are enzymes, which are essential in DNA replication and RNA transcription. The presence of anti-topoisomerase I antibodies is a highly specific feature of diffuse systemic sclerosis (SSc). Their role in the pathogenesis of SSc remains unknown. We have investigated the expression of the antigenic protein ,topoisomerase I, gene in cultured dermal fibroblasts from SSc patients by Northern blot analysis and semiquantitative polymerase chain reaction. Fibroblasts from SSc patients were found to express slightly increased amounts of mRNA for topoisomerase I, as compared to healthy individuals. In both, healthy controls and SSc patients, the expression of this gene was further up-regulated in the presence of Transforming Growth Factor β1 or Tumor Necrosis Factor α, i.e., cytokines abundant in SSc skin. These results may indicate a role of topoisomerase I in the pathogenesis of SSc and might suggest usefulness of topoisomerase I inhibitors in the treatment of SSc patients.

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LOCALIZATION OF ALPHA 3 INTEGRIN MESSENGER RNA IN CUTANEOUS SQUAMOUS CELL CARCINOMA.
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Alpha 3 integrin antigen has been shown to exhibit a peripheral pattern in invasive squamous cell carcinoma (SCC) tumor islands. In order to better define this characteristic expression of antigen, we examined the expression of alpha 3 mRNA sequences by in situ hybridization.
Parafin sections of invasive SCCs were hybridized with a 26 mer biotinylated single stranded probe to integrin alpha 3 mRNA. The signal was amplified using avidin biotin complex/alkaline phosphatase and read using a light microscope.
In tumor masses mRNA alpha 3 message was strongly exhibited centrally and decreased peripherally. Individual cells showed cytoplasmic localization of mRNA with increased expression near the cell membrane. Intercellular bridges were also markedly positive.
The pattern of expression of alpha 3 mRNA in SCC tumor islands corresponds to the pattern seen immunohistochemically in sections stained with monoclonal antibody P1B5 aganist the alpha 3 epitope. Localization of mRNA in the cytoplasm suggests accumulation of message which could readily be translated when signal is transduced. Finially mRNA in the desmosomes may indicate the transfer of message between cells for regulatory purposes.

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EXPRESSION OF TYPE VI COLLAGEN AND TENASCIN IN A NOVEL IN VITRO WOUND HEALING MODEL OF THE SKIN.
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Type VI collagen and tenascin are extracellular matrix proteins which appear during wound healing. Less is known about their function. A new in vitro wound healing model has been created to investigate their role in a system with epithelial-mesenchymal interaction: organotypic skin cultures under serum-free conditions have been wounded by sterile incisions or by punch biopsies, the defect has been filled with a fibrin matrix supplemented with different cytokine concentrations. Immunohistochemical investigation and in situ hybridization have been employed to evaluate the system over a two week period.

Essential processes of wound healing, the defect itself, release of cytokines, cell migration into the wound, proliferation, collagen synthesis, contraction, re-epithelization, can be simulated by this model. Thus this model helps to reduce animal experiments.

Transforming growth factor-β_1 stimulates mRNA and protein expression of type VI collagen and tenascin in a concentration and time dependent manner. Fibronectin and type III collagen also appear in the model wound. Type VI collagen, similar to type III, fills the wound with a fine mesh promoting a faster and more precise cell migration. Tenascin as an anti-adhesion molecule seems to facilitate the migration of fibroblasts into the wound.

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UPREGULATION OF INTEGRINS ON EPIDERMAL KERATINOCYTES AFTER IRRITATION OF THE SKIN. Peter von den Driesch, Anette Hüner, and Manige Fartasch

The basal layers of the epidermis constantly express different integrin heterodimers as $\alpha 2/\beta 1$, $\alpha 3/\beta 1$, $\alpha 6\beta 4$, and $\alpha_v\beta 5$. These cell-matrix adhesion receptors are suggested to contribute to the epithelial attachment to the basement membrane, but have also been localized at sites of cell-cell attachment between keratinocytes. Upregulation of these integrins has been demonstrated in a variety of inflammatory dermatoses such as psoriasis or eczema. The time-course of this modification however has not been elucidated so far. Using specific monoclonal antibodies we investigated normal skin of healthy volunteers (n=20) after irritation with 1% sodiumdodecylsulphate (SDS) for 1,2,3,4 and 24 hours. Upregulation of ß1, ß4, $\alpha 2$, $\alpha 3$, $\alpha 6$, and α_v integrin chains was demonstrable as soon as 3 hours after irritation and further increased in the 4 and 24 hours biopsies. Our results demonstrate that overexpression of epidermal keratinocyte integrins occurs early in an inflammation. This phenomenon may be due to local cytokine release and interpreted as an increase of keratinocyte cell-cell and cell-matrix adhesion interactions probably in order to enhance the epithelial stability and protective functions.

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RETINOIC ACID PRODUCES DERMAL COLLAGEN REPAIR IN PHOTOAGED HUMAN SKIN. J. J. Voorhees, A. N. Russman, C. E. M. Griffiths. Department of Dermatology, University of Michigan, Ann Arbor, MI.

Topical retinoic acid (RA) improves certain features of photoaged human skin, notably fine wrinkles. However, no observed histologic changes can account for this improvement. In the ultraviolet-irradiated mouse model of photoaging, effacement of fine wrinkles by RA correlates only with dermal collagen synthesis and not with histologic changes. Therefore, we investigated whether collagen synthesis was reduced in photoaged human skin and, if so, could it be restored by treatment with RA.

Skin biopsies from extensor forearm (photoaged) and buttock (sun-protected) were stained with a monoclonal antibody (SP1.D8) raised against the aminopropeptide of procollagen I. This antibody recognizes procollagen I within fibroblasts, and extracellular pN collagen I and aminopropeptide. Extent of immunostaining was assessed from 0, none to 5, maximum. Severity of photoaging in biopsied forearms was graded from 0, none to 9, most severe. Biopsies of photoaged skin from subjects treated with either 0.1% RA (n = 15) or vehicle cream (n = 14) were assessed for immunostaining at baseline and after 10 - 12 months treatment.

Immunostaining in sun-protected skin was observed extracellularly (pN collagen I) in a papillary dermal band and intracellularly within fibroblasts (procollagen I). In photoaged skin extracellular staining was reduced 56% compared to sun-protected skin (1.2 ± 0.2 vs. 2.7 ± 0.3 respectively, n = 26, $P < 0.0001$). Reduced extracellular, pN collagen I staining correlated with increasing severity of photoaging ($r = -0.58$, $P = 0.002$). RA treatment of photoaged skin increased extracellular pN collagen I by 80% (1.1 ± 0.2 to 2.0 ± 0.3) vs. a 14% decrease with vehicle (1.6 ± 0.3 to 1.3 ± 0.3, $P = 0.006$). RA treatment also increased fibroblast procollagen I staining by 119% (1.3 ± 0.3 to 2.9 ± 0.3) vs. an 18% decrease with vehicle (1.5 ± 0.2 to 1.3 ± 0.3, $P < 0.001$).

In summary, dermal damage in photoaged human skin is characterized by a significant reduction in collagen I formation which is partially restored by RA. These results imply that improvement of photoaging by RA is not simply cosmetic but rather is dependent on repair of dermal collagen.

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DIFFERENTIAL EFFECTS OF TUMOR NECROSIS FACTOR-ALPHA ON CHEMOTAXIS, GROWTH, COLLAGEN SYNTHESIS AND NONCOLLAGENOUS PROTEIN SYNTHESIS IN KELOID AND NORMAL SKIN FIBROBLASTS. Patricia A. Hebda, Margaret Collins, Robert Rodgers, Libbyette Wright and Michael D. Tharp, Department of Dermatology, University of Pittsburgh Medical Center, Pittsburgh Pennsylvania, USA

Tumor necrosis factor-α (TNF-α) is a pleiotrophic cytokine stored and secreted by macrophages and mast cells, both of which cell types are thought to be important participants in wound healing. Mast cells also have been found in increased numbers in the early inflammatory stage of scleroderma and at the margins of keloids. Therefore, mast cells have been implicated in the pathogenesis of cutaneous fibrotic disorders. The purpose of this study was to investigate the effects of TNF-α, the predominant mast cell-derived cytokine, on keloid and normal human skin fibroblasts. Cultures were established from surgically excised tissue; experiments were performed using cells in passages 3-6. The results suggest an overall inhibition of keloid fibroblasts by TNF-α: TNF-α was chemotactic and mitogenic for normal fibroblasts but not keloid fibroblasts; collagen synthesis was decreased for both but to a greater extent in keloid fibroblasts; and, noncollagenous protein synthesis was unaltered in normal but decreased in keloid fibroblasts. These observations suggest that mast cells may act to contain or "wall off" keloid lesions, releasing TNF-α locally to down-regulate keloid fibroblasts and promote the growth of the normal fibroblast phenotype.

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PCR-RFLP ANALYSIS OF MUTATIONS IN XPAC GENE IN JAPANESE PATIENTS WITH XERODERMA PIGMENTOSUM GROUP A. Kanokvalai Kulthanan[1], Chikako Nishigori[2], Hiraku Takebe[3], Shin-ichi Moriwaki[2], and Sadao Imamura[2], 1;Institute of Dermatology, Bangkok, Thailand, 2;Department of Dermatology, and 3;Experimental Radiology, Kyoto University Faculty of Medicine, Kyoto, Japan.

Forty-six Japanese patients with xeroderma pigmentosum group A were studied to determine the occurrence of 3 different mutations in XPAC gene. The first mutation was a splicing mutation of intron 3, detected by PCR-RFLP with AlwN I. The second was a nucleotide transition in exon 6 altering the Arg-228 codon(CGA) to a nonsense codon(TGA), detected by PCR-RFLP with Hph I. The third was a nucleotide transition in exon 3 altering the Tyr-116 codon(TAT) to a nonsense codon(TAA), detected by PCR-RFLP with Mse I. Of 46 cases studied, there were one case of homozygous Hph I-RFLP, two cases of compound heterozygote of AlwN I-RFLP and Hph I-RFLP, one case of compound heterozygote of AlwN I-RFLP and Mse I-RFLP. One patient (XP2NI) had splicing mutation and another mutation at the last codon of exon 5. The others were homozygous AlwN I-RFLP. There were no skin tumors detected in groups that were not homozygous AlwN I-RFLP. These data indicate that different genetic alteration in XPAC gene may induce difference in clinical features.

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TARGETED CYTOGENETIC ANALYSIS OF MALIGNANT MELANOMAS BY IN SITU HYBRIDIZATION WITH A SET OF CHROMOSOME-SPECIFIC DNA PROBES. Mayumi Matsuta[1], Yuko Imamura[1], Morimasa Matsuta[2], Saiichi Kon[1], Kosuke Sasaki[3], Department of Dermatology[1], Obstetrics and Gynecology[2], and Pathology[3], Iwate Medical University School of Medicine, Morioka, Japan

Fluorescence in situ Hybridization (FISH) with chromosome-specific repetitive DNA probes was applied for cytogenetic study of four malignant melanomas. A set ofsatellite DNA probes of chromosome 1,6,7 and 17 was applied on freshly isolated tumor cells in order to detect numerical chromosome aberrations of these chromosomes. Normal human lymphocytes as diploid cells and a malignant melanoma cell line RPMI served as controls. Furthermore, DNA analysis of these tumors was carried out by Flow Cytometry (FCM). FCM revealed that two cases of tumors showed the diploid pattern, and another two cases showed the aneuploid pattern. However, heterogenous distribution of centrometric copy numbers was clarified: two cases were chromosomal diploid of chromosome 6 and 17, and triploid of chromosome 7 and 1 by means of FISH. Another one showed a contratry pattern by FISH, and last one revealed one or many copies in interpahse nuclei. These data indicated that in situ hybridization with chromosome-specific DNA probes can serve a powerful tool for cytogenetic analysis of chromosomal aberration in human solid tumors.

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LINKAGE TO ANONYMOUS MARKERS AT 3p21 AND COL7A1 RFLPs IN 5 BRITISH FAMILIES WITH AUTOSOMAL DOMINANT DYSTROPHIC EPIDERMOLYSIS BULLOSA ASSOCIATED WITH ABNORMAL ANCHORING FIBRILS. L. Al-Imara, A.J. Richards, *R.A.J. Eady, *M.G.S. Dunnill, and F.M. Pope. Dermatology Research Group, Clinical Research Centre, Harrow, London and *Dept of Cell Pathology, St John's Institute of Dermatology, St Thomas' Hospital, London, England.

We recently described linkage to the anonymous marker D3S2 at 3p21 in three British families with dominant dystrophic epidermolysis bullosa (DDEB). We have now extended these studies in a further two families and also are confirming linkage with the COL7A1 PvuII RFLP. In addition we have studied the distribution of anchoring fibrils in affected individuals from each family. The combined lod score with the D3S2 marker currently exceeds $\theta = 9.65$. In four of the five families the DDEB phenotype segregates with the rarer + allele. The disease in the family segregating with the - allele has arisen from a new dominant mutation suggesting the possibility of a common founder effect in the other families. We are currently rechecking the lod score using the COL7A1 PvuII RFLP. Electron microscopy shows a consistent reduction in the numbers of anchoring fibrils in affected individuals and is consistent with the linkage data implicating COL7A1 as the mutant gene.

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EXCLUSION OF CANDIDATE GENES IN DARIER'S DISEASE USING POSITIONAL CLONING. L.A. Goldsmith, P. Wakem, R. Polakowska, A. Haake, N. Ewing, Y. Sarret, A. Trattner, B. Shohat, M. David, W. Schroeder, M. Duvic, S. Ikeda and E. Epstein, Jr. Departments of Dermatology, University of Rochester, Rochester, NY, USA; University of Lyon, Lyon, France; Tel Aviv University, Tel Aviv, Israel; University of Texas, Houston, Texas, USA; University of California, San Francisco, USA.

Using positional cloning techniques we have excluded many of the likely candidate genes implicated as the basic defect in Darier's disease (DD). Several large pedigrees from the USA, France and Israel were studied. Southern analysis of Restriction Fragment Length Polymorphisms were used for some loci; microsatellite repeats which mapped closely to other potential loci were used in other cases. All statistics were analyzed using the LINKAGE program with an estimated penetrance of 0.95 and a frequency for DD of 0.001. Loci with LOD scores <-2.0 include those for type I and type II keratin loci, the epidermal growth factor receptor, the alpha and beta-retinoic acid nuclear receptors, epidermal surface antigen-1 and the epidermal transglutaminase 1 gene.

Having eliminated likely candidate genes in DD it was reasonable to begin a genome-wide exclusion, using microsatellite markers. Preliminary results from those studies have eliminated chromosome 14p as a site of the DD gene. Further results using this positional cloning approach will be reported.

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POLYMERASE CHAIN REACTION FOR THE DETECTION OF MYCOBACTERIUM LEPRAE IN SKIN TISSUE, Kowit Kampirapap, Niwat Montreewasuwat, Maeya Ngamying, Sasakawa Research Building, Raj-Pracha-Samasai Institute, Nonthaburi 11000, Thailand

We have tested several sets of primers for the amplication of a 530 bp, 360 bp and 372 bp fragment of genes specifically encoding for 36 kDa, 18 kDa and 65 kDa protein of M.leprae respectively. All were found to be very specific and sensitive for the detection of M.leprae organisms in suspensions. We have used the primers S 13, S62 which amplified a 530 bp fragment of the 36 kDa protein gene of M.lepare in PCR technique on fresh and paraffinized skin sections. The results showed that PCR is much more powerful in specfic determination of M.leprae organisms in skin tissue as compared with conventional acid-fast staining method. Hybridization of the amplified fragments with digoxigenin-labelled 1 kb 36 kD gene probe increased the sensitivity of detection by 30%. A quantitative test to determine the proportion of viable bacilli in biopsy specimens by the PCR as compared with mouse footpad inoculation test is being carried out.

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GENETIC AND IMMUNOCHEMICAL ANALYSIS OF MUTANT P53 IN HUMAN MALIGNANT MELANOMA CELL LINES. Yukio Yamashina, Haruhiko Ohno, Masami Yamaji, Nobuhiko Kobayashi, Tsutomu Muramatsu, Toshihiko Shirai, Hideki Matsumoto*, Kumio Okaichi** and Masamitsu Ichihashi***, Department of Dermatology, 1st Department of Anatomy* and Department of Biology**, Nara Medical University, Kashihara and Department of Dermatology, Kobe University***, Kobe, Japan

Recently, the expression of the tumor suppressor gene p53 is analyzed in various human malignant tumors and the mutation of the p53 gene is reported in many tumors. Then the expression of the p53 protein in various malignant skin tumors is also reported.

In the present study, we examined the mutation of p53 genetically and immunochemically in 6 human malignant melanoma cell lines (HMV1, HM6KO, G361, P39, P22 and Mewo). In Western blotting analysis using an anti-p53 monoclonal antibody (PAb1801) with BLAST system for amplification, HM6KO showed the strong immunoreactant but other cell lines did not show positive reaction. For sequencing analysis of genomic DNA from these 6 melanoma cell lines, the regions exon 5 to 9 of the p53 gene were amplified into 3 fragments. From the analysis of the PCR products, we did not detect the homozygous deletion of the exons. Therefore, it is suggested that HM6KO has mutation of the p53 gene and synthesizes abnormal p53 protein.

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EXPRESSION OF FAR-17a, AN ANDROGEN-REGULATED mRNA IN THE SEBACEOUS GLANDS AND ITS CHARACTERIZATION. Toshihiko Seki, *Joseph A. Rothnagel, Hirofumi Aoki, Ritsuro Ideta, *Donnie S. Bundman, *Dennis R. Roop and Kenji Adachi, Adachi Research Laboratories, Shiseido Research Center, Yokohama, Japan ; *Department of Cell Biology & Dermatology, Baylor College of Medicine, Houston, TX, U.S.A.

Flank organs of Golden Syrian hamsters are useful for studying androgen-dependent growth of hair follicles and sebaceous glands. The flank organs are paired-pigmented spots on their backs, which consist of large sebaceous glands, hair follicles, and dermal pigment. After castration of male hamsters, the size of the flank organ decreases and subsequent androgen administration restores their normal size. To elucidate the mechanism of androgen regulation on gene expression in sebaceous glands and hair follicles, we constructed a cDNA library from flank organs of male hamsters and screened by a differential hybridization method using cDNA probes from normal and castrated males. We isolated a cDNA clone, termed FAR-17a, whose expression was found to be highly sensitive to androgen. FAR-17a mRNA of 1.8kb was reduced after castration and reappeared after testosterone administrations. Among several examined, FAR-17a gene was expressed at a high level in flank organ and low level in testis and earlobe. Also the expression of FAR-17a was detected in skin by reverse-transcribed PCR (RT-PCR). Its expression in skin was also androgen-regulated. FAR-17a probe detected a few fragments in genomic DNA of some other species including human, suggested that this gene is phylogenetically conserved. The sequence of FAR-17a cDNA predicts a protein of 231 amino acids (M.W. ~27,000 daltons) having basic properties. The deduced protein has no significant homologies to proteins previously described. We produced two different monospecific antibodies against the deduced FAR-17a protein, which were purified by affinity chromatography. These anti-FAR-17a antibodies detected protein of the same size by Western blot analysis and stained specifically the sebaceous glands of flank organ confirming the presence of FAR-17a protein in it. In order to examine whether the gene expression of FAR-17a is directly induced by the androgen receptor, we have isolated the genomic DNA of FAR-17a. Studies are underway to determine its sequence.

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DISTRIBUTION OF POLY-A mRNA IN NORMAL HUMAN SKIN. Takuji Masunaga[1,3], Shin-ichi Takahashi[2], Hiroshi Shimizu[1] and Takeji Nishikawa[1], [1]Department of Dermatology, Keio University School of Medicine, Tokyo, [2]Department of Dermatology, Ichikawa General Hospital of Tokyo Dental College, Chiba, [3]Research Laboratory, KOSÉ Corporation, Tokyo, Japan.

The amount and distribution of mRNA may reflect the cell activity. To investigate the distribution of mRNA in the skin is important to clarify the skin biology and pathophysiology. However, there are few reports concerned with the distribution of poly-A mRNA in the skin, and its distribution is not yet established. In this study, we attempted to find out the optimal condition to demonstrate the distribution of poly-A mRNA in normal human skin by *in situ* hybridization. Normal human skin was snap frozen and embedded in OCT compound, and stored in liquid nitrogen until use. The 6-μm-thick sections were fixed in 4% paraformaldehyde. After various pretreatments were applied, the sections were hybridized with digoxigenin labelled poly-dT (30mer) probe. Hybridized probe was immunohistochemically detected by using sheep anti-digoxigenin antibody conjugated to alkaline phosphatase. As a pretreatment, a combination of proteinase K (33 ng/ml, 15 min) and acetylation with acetic anhydride (0.25 v/v%, 15 min) gave the highest signal noise ratio. In all epidermal cells including granular, spinous and basal layer, the positive signal was equally observed in the nucleus and perinuclear space. The nucleus and cytoplasm of skin appendages were stained more strongly than those of epidermal cells. The all signals were completely disappeared by RNase treatment. Contrary to the previous report that a gradient of signal was observed with low amounts in basal layer, our results indicate that poly-A mRNA is expressed uniformly in the entire epidermis and expressed more strongly in the skin appendages. The distribution pattern which is different from that in other tissues indicates that the skin might undergo different regulation from other tissues.

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EFFECT OF ULTRAVIOLET LIGHT B IRRADIATION ON EPIDERMAL GROUTH FACTOR RECEPTOR GENE EXPRESSION. Naoki Maekawa, Michio Fukuda, Takeshi Kono, Hiroyo Fushida, Masamitsu Ishii, Toshio Hamada and Syuzo Otani*, Departments of Dermatology and *Biochemistry, Osaka City University Medical School, Osaka, Japan

Epidermal growth factor receptor (EGFR) plays a key role in the control of cell proliferation, and it is important to understand the factors which control its expression. We investigated effects of ultlaviolet light B (UVB) irradiation on the expression of EGFR in skin of mice by Northern blot hybridization and in situ hybridization. EGFR mRNA levels varried by UVB irradiation. In situ hybridization showed grains corresponding EGFR mRNA mainly in epidermis, especially in folicular epidermis. These results suggest that UVB irradiation is one of the factors which control EGFR expression.

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ISOLATION AND CHARACTERIZATION OF MOUSE DIFFERENTIATION-SPECIFIC SPR GENES (cDNAs).

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Spr genes were originally isolated from a human epidermal cDNA library as UV-inducible (T. Kartasova and P. van de Putte, Mol. Cell Biol.8: 2195-2203, 1988). Subsequently, it was shown that the expression of these genes is also induced by TPA and during epidermal differentiation. Mouse homologues of human spr genes were isolated 1) for the experimental carcinogenesis studies in a mouse skin model; 2) to get insight on the evolution of these genes in the animal kingdom.

Complete sprI and sprII cDNAs were isolated from a mouse papilloma cDNA library. Sequence comparison showed that the mouse spr genes were 60% larger than their human homologues due to a larger number of the conserved repeats. The overall structure was preserved between mouse and human spr genes. Northern blot analysis of RNA isolated from mouse embryonal skin revealed induction of the spr gene expression on day 16 which correlated closely with the induction of stratification in fetal skin. Antibodies were raised in rabbits using synthetic peptides corresponding to the N- and C-termini of the sprI and sprII proteins and purified by affinity chromatography. The specificity of these antibodies is currently being tested by western blotting and by immunohistochemical staining using mouse and human samples.

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POSITIVE AND NEGATIVE ELEMENTS INVOLVED IN THE TRANSCRIPTION OF THE HUMAN PROFILAGGRIN GENE. S. Jang, N.G. Markova, and P.M. Steinert, Skin Biology Branch, NIAMS, NIH, Bethesda, Maryland, U.S.A.

Human profilaggrin is a major epidermal intermediate filament-associated protein. It is synthesized in the late stages of epidermal differentiation. The level of expression is calcium dependent, and in cultured keratinocytes, profilaggrin mRNA transcription can be induced with calcium. Recently we characterized the human profilaggrin transcription unit and especially the 5' region where most of the regulatory signals are normally located. The proximal and distal promoter regions contain many putative regulatory elements, some of which have been implicated in the regulation of other epidermally expressed genes. To elucidate the regulation of transcription of profilaggrin mRNA, a series of fragments encompassing gradually increasing portions of the 5'-region of the profilaggrin gene were cloned into a CAT-reporter vector and used in a transient expression assay. The CAT expression was low in epithelial cells (HeLa) and undifferentiated epidermal cells, such as RHEK and cultured normal human epidermal keratinocytes (NHEK) grown in low calcium. When calcium concentration was raised to 1.2 mM in NHEK, different degrees of CAT expression were observed from each construct. Similar profiles of CAT expression were found in HaCat cells. The construct containing first 120 bp upstream of the transcription initiation site exhibits a significant enhancer activity. Bandshift experiments with NHEK and HaCat cellular extracts reveal that at least three proteins bind to regulatory sequence sites in this proximal promoter region confirming a differentiation specificity to the expression. An enhancer element is situated between positions -359 and -705. Three negative elements are located between positions -120 and -359, -705 and -1500, and -1500 and -3600, respectively. Further band shift experiments and DNA footprinting analyses will now provide a detailed characterization of these regulatory sequences.

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PROTEIN SYNTHESIS INHIBITORS INDUCE INTERCELLULAR ADHESION MOLECULE-1 GENE EXPRESSION IN NORMAL AND TRANSFORMED CELLS

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Our previous study demonstrated that protein inhibitor like cycloheximide (CHX) induced intercellular adhesion molecule-1 (ICAM-1) mRNA in human squamous cell carcinoma (SCC) cell lines. This present study further examined using Northern blot analysis cultured human normal cells and various human transformed cells in terms of the effects of protein synthesis inhibitor with or without interferon-γ (IFN-γ) on ICAM-1 mRNA induction. Not only normal keratinocytes, endothelial cells, dermal fibroblasts, but cervix carcinoma cells (HeLa), epidermoid carcinoma cells derived from the larynx (HEp-2), hepatocellular carcinoma cells (Hep G2), melanoma cells (HMV-1), and monocytic leukemia cells (U937, THP-1) induced ICAM-1 mRNA by the protein inhibitor as well as IFN-γ. These results suggested that the presence of cellular factor which suppress ICAM-1 gene were not only found in SCC cell lines, but also in many other various cells.

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NUCLEOSIDE DIPHOSPHATE KINASE EXPRESSION IN HUMAN SKIN. Yoshio Urano and Seiji Arase, Department of Dermatology, School of Medicine, The University of Tokushima , Tokushima, Japan

Recent studies have demonstrated the presence of nucleoside diphosphate (NDP) kinase in association with proteins presenting strict specificity for guanine nucleotides, including microtubules, initiation factor eIF2, and G proteins, suggesting that NDP kinase may play a role in many cellular events by replenishment of nucleoside triphosphates (NTPs) in the immediate vicinity of the NTP-requiring reactions. Few studies on NDP kinase expression in human skin have been performed so far. In this study, we examined NDP kinase expression in human skin using a rabbit polyclonal antibody against rat liver NDP kinase.

An immunoblot analysis using whole skin extracts detected a 19 kDa band. Its size corresponded to that of rat liver NDP kinase. Immunohistochemical analyses showed the presence of NDP kinase in the epidermis, hair follicles, and eccrine and apocrine sweat glands. In the epidermis, basal and suprabasal cells were more strongly positive than spinous cells. In the outer root sheath of a hair follicle, the outermost layer was more deeply stained than inner layers. In the eccrine sweat duct, basal ductal cells were more strongly positive than luminal cells. These findings suggest that NDP kinase is expressed more abundantly in metabolically active cells of human skin.

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CALRETICULIN (CR) IS TRANSCRIPTIONALLY REGULATED BY HEAT SHOCK. T.Q. Nguyen, J.D. Capra, R.D. Sontheimer. Depts. of Dermatology and Microbiology, U.T. Southwestern Med. Center, Dallas, TX, 75235.

CR has recently been confirmed to be a new human rheumatic disease-associated autoantigen. In our studies, this 46 kD (60 kD in SDS-PAGE) high-affinity calcium-binding protein of unknown function is physicially associated with the Ro/SS-A autoantigen complex. Since cellular modulation of Ro/SS-A antigen expression has been implicated in the pathogenesis of subacute cutaneous lupus erythematosus (LE) and neonatal LE, we have begun to examine the genetic regulation of CR. To examine transcriptional regulation of CR, a reporter gene construct was developed by inserting a 511 bp fragment of the 5' flanking region of a genomic clone of CR into a plasmid containing a bacterial chloramphenicol acetyltransferase (CAT) reporter gene (CR-CAT). CR-CAT was then transfected into A431 cells and selective growth in G418 was used to isolate three clones that were confirmed by polymerase chain reaction and Southern blotting to have CR-CAT stably integrated into genomic DNA. Each of the three clones was found to constitutively express low levels of CAT activity as detected by thin layer chromatography. Calcium ionophore shock (A23187 - 2.5 μM/ml), heat shock (43°C x 1 hr) and zinc ion exposure (225 μM) were found to increase CAT expression in these clones by 800-1,200%, 400-600% and 500-1,700% respectively. Studies are underway to examine the effects of ultraviolet B light on CR-CAT expression. These results suggest that a) the 511 bp fragment of the 5' regulatory region examined in this study is sufficient for promoting transcription of the human CR gene, b) CR is constitutively transcribed in a transformed epidermal keratinocyte line, c) CR is regulated at the transcriptional level, and d) CR, like several other LE-related autoantigens, appears to function as a heat shock gene.

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ACTIVITY OF VIRAL PROMOTORS IN CULTERED HUMAN SKIN CELLS. Metin Artuc, Wolf Nürnberg, Matthias Platzer*, Beate M. Czarnetzki and Dirk Schadendorf, UKRV, Dermatology, and Max-Dellbrück-Center for Molecular Medicine(*), FU Berlin

Promotor and enhancer activities that control gene transcription vary considerably among different cell types. The combination of different recognition sequences and the amounts of cognate transcription factors determine the efficiency with which a given gene is transcribed in a particular cell type. In order to develop systems to transiently or stablly express mammalian proteins in human skin-derived cells, we tested 7 different viral promotors (pCMV, pRSV, pHMT, pPMTV, PUPE, PUPL, pSVE) to drive the expression of the chloramphenicol acetyltransferase (CAT) enzyme in epithelial cells (HT-3), human normal keratinocytes and neuroectodermal cells (SK-Mel 23, SK-Mel 37). DNA was transfected using a liposome-based technique and transfection efficacy was controlled by co-transfection of a ß-galactosidase gene construct. The enzymatic activity of the CAT-gene expression was determined by incubation of the cell extract prepared from transfected cells with ^{14}C-labeled chloramphenicol. CAT activity was correlated to the ß-galactosidase activity and protein amount. In conclusion, strategies for overexpression of foreign genes in human epithelial and melanocytic cells should consider the usage of the CMV promotor, since only the viral replicon of CMV and less so of RSV were active in our cell-line tested. The expression plasmid containing the SVE promoter was only active on the epithel cell-lines tested and seems to be of sufficiently value in studing gene expression in human epidermal cells.

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STUDY OF STEROID SULFATASE GENE DELETIONS AND LETECTION OF THE CARRIERS Zhi-lan Hu and Li Fang, Department of Dermatology, Hua Shan Hospital, Shanghai Medical University, Shanghai, P.R.China

Recessive X-linked Ichthyosis(RXLI) is one of the most common X-linked genetic disorders, which is attributed to deficiency of steroid sulfatase(STS). In approximately 90% of the patients, this lack is due to the deletion of STS gene which is located at Xp22.3. The cDNA probe of STS gene has been isolated previously by other groups. For the first time in China, we analyzed 19 cases of RXLI and 11 cases of Autosomal Dominant Ichthyosis(ADI) by Southern blot hybridization and Polymerase Chain Reaction(PCR) as well. We found that 84% (16 out of 19) RXLI have STS gene deletions while ADI haven't any. Furthermore, as the carrier has a half-normal gene dosage, we have used gene dosage analysis to detect the carriers in four RXLI families and found two mothers be heterozygote. These experiments contribute to rapid, simple and effective gene diagnosis and prenatal diagnosis of RXLI.

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XENOBIOTICS ARE IMPORTANT IN SKIN TUMORIGENESIS: EVIDENCE FROM STUDIES OF GLUTATHIONE S-TRANSFERASE GENOTYPE DELETION. Adrian Heagerty, Anthony Fryer, Julie Alldersea, Lei Zhao, Andrew Smith, William Bowers', Richard Strange.School of Postgraduate Medicine, Keele University, The North Staffs Hospital Centre, Stoke-on-Trent, and 'Dept Dermatology The Royal Cornwall Hospitals, England.

Although excessive UV light is considered the major causative agent in the pathogenesis of skin tumours, xenobiotics such as cyclic hydrocarbons have been implicated. Detoxication of some of these is reliant on elimination after conjugation with glutathione. The efficiency of detoxication of such potential carcinogens may, therefore, be a factor determining susceptibility to these cancers. Thus, deletion of the phase 2 detoxicating enzyme glutathione S-transferase M1 (GSTM1), is associated with increased risk of developing certain lung, pituitary and gastrointestinal tumours. Using primers to exon 4/5 to identify deletion of GSTM1 (GSTM1 0) and to intron 6/exon 7 to identify the A and B alleles, we examined the genotypes of 481 patients with skin tumours, comprising 314 patients with basal cell carcinoma (BCC), 72 with squamous cell carcinoma and Bowens disease (SCC) and 62 with malignant melanoma (MM), as well as a group of 33 patients each with at least two different tumours. Patients with homogeneity of tumour type, even when multiple, had no difference in the incidence of the deletion compared with controls. Patients with multiple different tumours, however, showed a significantly increased incidence of the null genotype (X^2=6.33, OR 3.28, CI 1.26-8.67, p<0.012), indicating GSTM1 0 is a susceptibility marker for the development of multiple heterogenous skin tumours and that failure to metabolise certain xenobiotics is involved in tumorigenesis in the skin. Studies of allelic frequencies showed no preponderance of A or B in those patients without the deletion.

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ABNORMALITY OF P53 TUMOR SUPPRESSOR GENE IN NON-MELANOMA SKIN CANCER. Tohru Nagano, Masato Ueda and Masamitsu Ichihashi, Department of Dermatology, Kobe University School of Medicine, Kobe, Japan

P53, a tumor suppressor gene, has been suggested to play an important role for the carcinogenesis and differenciation of keratinocytes. To elucidate the role of p53 in non-melanoma skin cancer, paraffin sections from squamous cell carcinoma (SCC), solar keratosis (SK) and basal cell epithelioma (BCE) were immunohistochemically stained with the CM-1 antibody. In addition, some DNAs extracted were analyzed for the sequence of p53. By staining, 14/26 (54%) of UV-related SCCs were positive, whereas 5/26 (19%) of UV-unrelated SCCs were positive. 11/23 (48%) of SKs were positive. 3/21 (14%) of BCEs were positive. DNA sequencing of exon 5,7 and 8 of p53 from 8 BCEs revealed that 2 tumors had point mutation. A BCE from nevoid basal cell syndrome had a mutation at codon 273 of exon 8 (CGT to TGT). Another BCE had a point mutation at codon 135 of exon 5 (TGC to AGC), where is the di-pyrimidine site. These data indicate that p53 has a crucial role for the carcinogenesis of non-melanoma skin cancer and UV may be one of the mutagens for p53 gene.

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P53 GENE MUTATIONS IN HUMAN SKIN TUMORS: COMPARISON WITH AN IMMUNOHISTOCHEMICAL ANALYSIS. Yoshiaki Kubo, Yoshio Urano and Seiji Arase, Department of Dermatology, School of Medicine, The University of Tokushima, Tokushima, Japan

Inactivation of the p53 tumor suppressor gene is one of frequently observed major molecular etiologies for human tumorigenesis. In this study, mutations in exons 3 to 9 of the p53 gene were screened in 23 squamous cell carcinomas (SCCs) of the skin, 25 basal cell carcinomas (BCCs), two cases of Bowen's disease, 10 cases of solar keratosis and 5 keratoacanthomas by polymerase chain reaction-single strand conformation polymorphism analysis. Mutations of the p53 gene were detected in 7 of 23 SCCs (30%), 3 of 25 BCCs (12%), and none in Bowen's disease, solar keratosis, or keratoacanthoma. Five of 7 mutations detected in tumors developing in sun-exposed areas were C to T transition. Both of two mutations found in SCCs originating at scar tissues were C to A transversion and a mutation observed in one SCC related to radiation dermatitis was a double base change of CC to AT. In 12 SCCs, we compared the results of the DNA sequence analysis with those of an immunohistochemical analysis which were reported previously. Two of 4 cases with positive staining had missense mutations and the other 2 cases had no mutation. Three of 8 cases with negative staining had nonsense mutations and the remaining 5 cases had no mutation. We concluded that mutations of the p53 gene are often observed in SCC of the skin in Japanese patients and that both pseudo-negative and pseudo-positive cases exist in an immunohistochemical analysis with respect to p53 gene mutations.

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IMMUNOCYTOLOGICAL LOCALIZATION OF CDC2 IN SQUAMOUS CELL CARCINOMA OF THE SKIN. Shinichi Inohara, Eriko Okamoto, Yukio Kitano, Department of Dermatology, Hyogo College of Medicine, Nishinomiya, Japan

Central to the mechanism of cell cycle control is cdc2 kinase, activation of which induces phosphorylation of key proteins including p53 which regulate cell cycle. On the other hand, phosphorylation of p53 is found in some types of malignant cells in addition to mutations of p53 gene. So, cdc2 is thought to be concerned with proliferation and carcinogenesis. In this study, immunocytological localization of cdc2, p53 (antibody against p53 used in this study recognizes wild type p53), and PCNA (a marker of proliferating cells) were examined in serial sections of normal epidermis and squamous cell carcinoma (SCC) of the skin. Furthermore, similar immunocytological studies were also conducted in the culture of both normal epidermal cells and malignant epidermal cells from SCC. In all the normal epidermis examined, only PCNA was positive to the nuclei of some basal cells, and cdc2 and p53 were negative. With SCC of the skin, PCNA was positive to the nuclei in many cells in all the cases examined. p53 was positive to the nuclei of some cells in 2 out of 4 cases of SCC. cdc2 was positive to the nuclei of some cells in 2 out of 4 cases. These two cases were also p53-positive and the location of cdc2 was almost consistent with that of p53. With cultured epidermal cells, PCNA was found in the nuclei of almost all cells from both normal epidermis and SCC. Neither cdc2 nor p53 was found in normal epidermal cells but these were recognized in some malignant cells from SCC. These results suggest that cdc2 may play a role in carcinogenesis of epidermal cells probably in relation to p53.

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CARCINOGENICITY STUDY OF PHYTOL(3,7,11,15-TETRAMETHYL-2-HEXADECEN-1-OL) IN ICR MICE. Masayori Kagoura, Chihiro Matsui and Masaaki Morohashi, Department of Dermatology, Toyama Medical and Pharmaceutical University Faculty of Medicine, Toyama, Japan

Phytol(3,7,11,15-tetramethyl-2-hexadecen-1-ol), a kind of alcohol, is a part of chlorophyll. We studied the carcinogenicity of phytol on the skin of ICR mice and compared the promotion activity between phytol and TPA (12-o-tetradecanoylphorbol-13-acetate). We used 7-week-old female ICR mice. A single dose of 100μg DMBA(9,10-dimethyl-1,2-benzanthracene) was used as an initiator and was followed 1 week later by 0.12g phytol or 2.5μg TPA as promotor. The promotion treatment was given to all groups, 2 days a week forabout 4 months. As a result of the initiation/promotion protocol, 100% of the animals in the group treated with DMBA followed by TPA developed skin tumors. In the group treated with DMBA followed by phytol, 95% of the animals developed skin tumors. The average number of lesions per mouse treated with DMBA and TPA was significantly different from that of group treated with DMBA and phytol(P=0.01).

On histological examinations, we observed hyperplasia or papilloma of the epidermis at early stage. In some tumors, ulceration or erosion at the top of lesions gradually occured. Squamous cell carcinoma was the pathological diagnosis and there was partial differentiation of the hair follicles.

We suggest that phytol had weak a carcinogenic effect compared with TPA and the apperance of tumors with partial differentiation of the hair follicles were interesting findings.

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ECCRINE KERATINOUS CYST: A PROPOSAL FOR THE PATHOMECHANISM OF PALOMOPLANTAR EPIDERMOID CYSTS. Kiyofumi Egawa, Yumi Honda, Youichi Inaba, Kazuko Yoshimura and Tomomichi Ono. Department of dermatology, Kumamoto University School of Medicine, Kumamoto, Japan.

Although epidermoid cysts in palmoplantar locations have long been thought to develop after deep implantation of an epidermal fragment resulting from a penetrating injury, the true pathomechanism of this disorder remains unclear.

We examined 119 cases of the cysts histologically and immunohistochemically for the role of the eccrine sweat gland, as well as human papillomavirus(HPV), in the pathomechanism of this disorder. Histological divergences were noted between the cysts: three distinct histological features including intracytoplasmic eosinophilic bodies (IEB) in the cyst wall, vacuolar structures(V) and parakeratotic nuclei(P) in the cyst cavity were noted in 14 cysts; V and P were in 12 cysts; IEB and V in one cyst; only V in one cyst; only P in 59 cysts; and only keratinous mass in 31 cysts. Papillomavirus common antigens were detected in 36(30%) cysts showing at least one of the three histological features. Another histological feature suggesting eccrine sweat ducts or an expression of CEA/CA 50 was noted in 73(61%) cysts.

Based on our results, we propose cyst formation is induced from the eccrine ducts, while HPV and injury may play a role in the mechanism.

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DETECTION OF MESSENGER RNA FOR THE *TAX* GENE OF HUMAN T-LYMPHOTROPIC VIRUS TYPE I IN SKIN LESIONS OF ADULT T-CELL LEUKEMIA/LYMPHOMA BY USING *IN SITU* HYBRIDIZATION. Mitsuru Setoyama, Yoshihiko Katahira, Shimako Mizoguchi and Tamotu Kanzaki, Department of Dermatology, Faculty of Medicine, Kagoshima University, Japan

Adult T cell leukemia/lymphoma (ATLL) is a malignant lymphoproliferative disorder which involves skin as well as peripheral blood and lymphnodes. It is proposed to categorize into 5 clinical types; acute, crisis, lymphoma, chronic, and smoldering based mainly on data of patient's peripheral blood.

Our previous study proved the production of human lymphotropic virus type 1 (HTLV-I) antigens on tumor cells in short-term cultured skin lesions from an ATLL patient. However, HTLV-I antigen production has neither been detected in *vivo* by Western blotting nor Northern blotting studies. *In situ* hybridization is a technique that can identify cells that contain mRNA encoding for a particular protein.

The object of this study was to use a non-radioactive *in situ* hybridization technique to determine the presence of mRNA encoding for *tax* region of the HTLV-1 genome if it is present in ATLL cells *in vivo*.

We present results that mRNA coding for protein *tax* 40 is localized in the nuclei and cytpoplasms of lymphoid cells in skin lesions in 4 out of 6 ATLL patients. Furthermore, in 3 cases, the intensity of signals became increased after 24 hr cultivation in antibody-free medium. This is the first report of HTLV-1 mRNA detected in cells of ATLL skin lesions.

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A DIVERSITY OF IMMUNOBIOLOGIC FUNCTIONS OF HTLV-1-INFECTED T-CELLS AND CUTANEOUS LESIONS OF ADULT T-CELL LEUKEMIA. Keiji Iwatsuki, Hiroshi Harada, Fumio Kaneko, Department of Dermatology, Fukushima Medical College, Fukushima, Japan

Patients with adult T-cell leukemia(ATL) develop various cutaneous lesions and clinical courses despite the phenotypic similarity of the malignant clones. We have studied cytological profiles of five HTLV-1-infected cell lines established from patients with ATL in order to clarify histogenesis of the cutaneous lesions. All the cell line cells showed a phenotype of CD3+, 4+, 8-, 25+, ICAM-1+, HLA-DR+, and LFA-1+. Three out of the five cell lines spontaneously secreted both IL-4 and IFN-γ, and induced ICAM-1 on cultured keratinocytes, but the other clones did not. The former cell types except one adhered to the cultured keratinocytes more vigorously than the latter. When the culture was continued by changing concentrations of exogenous IL-2 in the culture media, both IL-2-dependent and -independent cells, which showed different morphology and cell sizes, appeared among the same cell line cells. These results suggest that a diversity of immunobiologic functions of HTLV-1-infected T-cells and further clonal selection among the same cell line are responsible for the development of various cutaneous lesions in patients with ATL.

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ANALYSIS OF A 15KD PROTEIN ASSOCIATED WITH HTLV-1 gp46. I. Göttfried, M. Mildner, C. Ballaun, D. Reiser and E. Tschachler, Division of Immunology, Allergy and Infectious Diseases, Department of Dermatology, University of Vienna Medical School, Vienna, Austria

The human T-lymphotropic virus type I (HTLV-I) was the first human pathogenic retrovirus to be isolated. It has been primarily connected with the pathogenesis of adult T cell leukemia but has also been found associated with a distinct neurological disorder i.e. HTLV-I associated myelopathy/tropical spastic paraparesis. Whereas CD4+ T lymphocytes appear to be the predominant target cells for HTLV-I *in vivo*, its *in vitro* host range is rather broad. A cellular receptor for this virus has not yet been identified.To study the mechanisms of HTLV-I infection we analysed the large envelope protein (gp46) of HTLV-I produced by infected cells lines. When we performed radioimmunoprecipitation we found a protein of about 15 Kd which coprecipitated with HTLV-I gp46 in 3 of 4 cell lines productively infected by HTLV-I. This 15 kd protein is not recognized by the rabbit anti-gp46 antisera directly since these sera readily recognized gp 46 yet failed to detect the 15 kd molecule by Western blot analysis. The fact that sera of 7 HTLV-I infected patients did not detect the 15 kd protein by Western blot analysis of immunoprecipitates of gp46 indicates, that this protein is of cellular origin rather than a viral gene product. Deglycosilation studies performed on radioimmunoprecipitates showed that in contrast to gp46 the 15 kd appears not to be N-glycosilated. Analysis by 2D gel electrophoresis revealed that 6 different isoforms of this 15 kd protein in the pH-range from 6.5 to 8.9 exist. To clarify the exact nature of this 15 kd protein which is associated with HTLV-I gp46 we are currently purifying it for protein sequencing.

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EXPRESSION OF PROLIFERATING NUCLEAR ANTIGEN (PCNA) IN BOWENOID PAPULOSIS IS INDUCED BY HUMAN PAPILLOMAVIRUS-16 E7 ONCOPROTEIN. T. ISHIJI[1,2,3], M. Honda[2], M. Niimura[2], H. Matsushima[3], K. Kikuchi[3] and S. Yasumoto[3], [1]Dept. of Dermatology, Atsugi Hospital, Kanagawa, Japan. [2]Dept. of Dermatology, The Jikei University School of Medicine, Tokyo, Japan. [3]Kanagawa Cancer Center Research Institute, Kanagawa, Japan.

Previous studies have demonstrated that bowenoid papulosis is associated with infection of type 16-related HPVs. By using in situ hybridization technique with HPV-16/18 mixture probe, HPV DNA was detected from bowenoid papulosis specimens. In order to examine proliferative activities of these cells, expression of PCNA was studied in the same specimens using immunohistochemical method with the monoclonal antibody. We detected significant amounts of PCNA accumulation in the HPV-infected cells. On the other hand, it is known that HPV-16 E7 gene induces cellular proliferation of human foreskin keratinocytes. For checking the ability of E7 gene product to induce PCNA expression, E7 expression vector was co-transfected with PCNA promoter-CAT reporter plasmid. E7 enhanced CAT expression from the PCNA promoter in some cell lines. These data suggested that expression of PCNA in HPV-16 infected cells could be induced by E7 protein.

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DETECTION OF HUMAN PAPILLOMAVIRUS ANTIBODY BY TRANSFECTED COS CELLS. Akihiro Saito, Masaaki Hamada, Michio Nakano, Masaaki Morohashi, Department of Dermatology, Faculty of Medicine, Toyama Medical and Pharmaceutical University Faculty of Medicine, Toyama, Japan.

Human papillomavirus (HPV) -1 and -2 are the most common types to induce benign papillomatous lesions, however it is very difficult to establish model systems to express the HPV genome. To develop an in vitro experimental system, COS cells were transfected with HPV-1a or -2a DNA, and selected for expression of HPV proteins using immunofluorescence and immunoprecipitation methods. Immunoprecipitation reaction of the transfected COS cells and the patient's serum allowed the detection of specific bands. It was suggested that this model expressed the HPV gene. We then attempted to confirm the reaction of the patient's serum to the transfected cells with an immunofluorescent study. The reaction was almost correlative to the clinical type. This system can be used for serological diagnosis and seroepidemiological studies of HPV infection.

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DETECTION WITH THE POLYMERASE CHAIN REACTION OF HUMAN PAPILLOMAVIRUS DNA IN CONDYLOMATA ACUMINATA TREATED IN VITRO CO_2 LASER, AND MICROWAVE. Hong-Xia Li[1], Wen-Yuan Zhu[2]. Reserch laboratory of Genetics Department, Nanjing Hospital for women[1], Department of Dermatology, Nanjing Medical College[2], Nanjing, P. R. China

The treatment of condylomata acuminata (genital warts) may be many forms. For example, CO_2 laser, liquid nitrogen, and microwave. But recurrent rates were highly. Reported recurrent rate for laser was 3—95 %. The recurrent reason is unknown. Our purpose was to determine wither CO_2 laser and microwave damage HPV DNA found in condylomata acuminata. Twelve specimens of genital warts were excised from 12 patients and divided into three parts. One part was untreated, the second and the third part were treated with CO_2 laser and microwave, respectively. The depth of the vaporization was judged with reference to the specimen char and the dull white. DNA was then extracted from tissue by proteolytic digestion and amplified by the polymerase chain reaction. HPV DNA was amplified and detected in 100% of untreated specimens (6-HPV 6 ; 6-HPV 11), in 83. 3% and 50% of specimens treated with CO_2 laser and microwave, respectively. There was a significant difference in detection between untreated and microwave-treated specimens ($X^2=4.18$, $P<0.05$). Microwave damages HPV DNA more effectively than CO_2 laser.

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HIRSUTOID PAPILLOMAS OF VULVAE: ABSENCE OF HUMAN PAPILLOMA VIRUS (HPV) DNA BY THE POLYMERASE CHAIN REACTION. Wen-Yuan Zhu, Ming-Yu Xia, Hong-Xia Li, Wei Wang and Zan Xu, Nanjing Medical College, P. R. China

Papules occuring on the inner surfaces of both labia minora are not rare and have been described under a variety of termis including pseudocondyloma of valvae and hirsuties papillaris vulvae. These papules clinically resemble the papules of genital condylomata. To study the possible association of HPV infection with hirsutoid papillomas of vulvae, we have analyzed specimens from 16 wemen with hirsutoid papillomas of vulvae for the presence of HPV DNA using the polymerase chain reaction. The subject's ages ranged from 27 to 43 years. In all cases, smooth or filiform papules were symmetrically located on the inner surface of both labia minora. Histologically, the lesions consist of a core of normal connective tissue covered by epidermis without koilocytes and mitotic activity. We used a primer set designed to amplify a 450 bp region of the L1 open reading frame. Positive controls included plasmids containing HPV sequences and appropriate negative controls were employed. HPV DNA could not detected in 16 tissue specimens of hirsutoid papilloma of vulvae. Positive controls present in each assay. Six of 16 specimens were studied by transmission electron microscopy. No HPV granules were found in the nuclei of keratinocytes. These results suggest that the papules of hirsutoid papilloma of vulvae are unrelated to HPV.

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KERATINOCYTE DIFFERENTIATION AND ITS EFFECT ON HPV REPLICATION. C J Sexton[1,2], A T Williams[1], J N Stables[2], K J Purdie[1], C Proby[1], I M Leigh[1], [1]Dept of Experimental Dermatology, London Hospital Medical College,[2]Wellcome Research Labs, Beckenham, UK.

Vegetative growth of human papillomavirus relies upon differentiation of its natural host cell, human keratinocytes, resulting in a complicated interplay of viral and cellular factors. We have used monolayer, raft, and xenograft systems to further our understanding of the role of keratinocyte phenotype and differentiation in the infectious cycle of HPV. Skin grafting techniques have been used to create an environment permissive for replication of endogenous HPV via the formation of stratified grafted epithelium. This xenograft model faithfully reproduces the gross morphological and histological features of HPV infection resulting in amplification of viral DNA, late gene expression, and the formation of macroscopic warts. Changes in cell proliferation markers, connective tissue, vascularization, and keratin/integrin expression have been monitored over a 3 month period compared to baseline wart tissue.

To study the interaction of HPV with basal cells in more detail, we have cultured anogenital and oral wart keratinocytes and examined the dynamics of episomal HPV maintenance in relationship to keratinocyte phenotype. Episomal HPV DNA has been found to persist for up to three passages with dramatic loss of episome within a single passage by slot blot hybridization. PCR analysis however revealed low copy number HPV in samples in which the episome was no longer detected by slot blot. Finally, we have examined the effects of soluble factors, cell trauma and virus infection upon keratinocyte growth and differentiation in HPV positive, negative, and SV40 Tag keratinocyte lines.

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VIRAL INTERFERENCE IN HERPES ZOSTER BETWEEN HSV AND VZV. Kiyoshi Kagami. Department of Dermatology , Kyoto First Red Cross Hospital, Kyoto,Japan

We noticed clinically that zoster's eruption was as apt to appear on the 1st branch area of n.trigeminis than 2nd and 3rd branches. Meanwhile, eruption of herpes simplex is often apt to appear in the oral cavity or on the lips. We know immunologically a cross reaction by CF antibody partially occurs between VZV and HSV. Therefore,we suspect that in the infection of HSV and VZV interference between them in the trigeminal ganglion by occupying viruses takes place. At first the infection of HSV occurs in the oral cavity and then HSV invades latently in the 2nd and the 3rd ganglions of n.trigeminis. Secondarily,the infection of the oral cavity by VZV as chicken pox occurred, and then VZV invaded the rest of the 1st ganglion of n.trigeminis latently. When VZV appeared again as herpes zoster, the lesion involved much more on the 1st branch area of n.trigeminis than on the 2nd and/or 3rd branch areas. To find out if this consideration was correct or not, we examined the frequency of lesional location on n.trigeminis. We examined the clinical charts of 12,239 out patients for 2 years in our hospital. There were 78 patients with trigeminal zoster lesions. There were lesions on the 1st branch of 50 cases, which is 64.1% of the total zoster appearance on trigeminal nerve,the lesions on the 2nd branch occurred in 17 cases the ratio being 21.8% of the whole, the lesions of the 3rd branch was found in 16 cases the ratio being 20.5% of the whole. At first the trigeminal 2nd and 3rd ganglions were occupied by HSV as latent infection, and secondarily,chicken pox occurred on the oral mucous membrane caused by VZV, the remaining trigeminal nerve (1st ganglion)was occupied also by VZV as latent infections and when zoster occurred, the trigeminal lesions on the first branch area were infected.

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DETECTION OF HERPES SIMPLEX VIRUS DNA IN CUTANEOUS LESIONS OF POST-HERPETIC AND PHOTO-INDUCED ERYTHEMA MULTIFORME. K. Yokoi, M. Honda, R. Kamide, M. Niimura and R. Hondo*, Department of Dermatology, The Jikei University School of Medicine, and *Department of Microbiology, Institute of Public Health, Tokyo, Japan.

Recently using the polymerase chain reaction (PCR), herpes simplex virus (HSV) DNA has been detected in up to 80% of the lesions of erythema multiforme (EM) whether they are associated with a preceding HSV infection or not. These high detection rates, however, are not coincident with clinical observations. In this study we used PCR to detect HSV DNA in cutaneous lesions from patients with post-herpetic erythema multiforme (PHEM) and photo-induced erythema multiforme (PIEM). DNA was extracted from deparaffinized sections of lesional skin from patients with PHEM (n=7) and PIEM (n=3) using the SDS/proteinase K method, amplified by PCR, and was then identified by the Southern blot hybridization method. We used two sets of primers. One (92-primers) had been used in all previous studies for detecting HSV DNA in EM lesions, amplifying a 92 bp segment corresponding to the HSV DNA polymerase gene (UL30). The other (847-primers), which was newly designed for DNA diagnosis of HSV serotypes, amplifies a 847 bp segment corresponding to the glycoprotein C-1 gene (UL44-UL45). While hybridization of the amplified products by 92-primers detected HSV DNA in 4 of 7 specimens of PHEM, none was detected in PIEM. However, none of specimens amplified by 847-primers was positive for viral DNA. These results support the association of HSV in the pathogenesis of PHEM and not in PIEM, but further studies are required to confirm the presence of the entire HSV genome in cutaneous lesions and investigate its etiological role in the pathogenesis of EM.

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CORRELATION BETWEEN CLINICAL FINDINGS AND VASCULAR INVOLVEMENT WITH LOCALIZED IMMUNE REACTION IN HERPES ZOSTER. Utsugi Iwasawa, Kaori Miyakawa and Hiroyuki Suzuki, Department of Dermatology, Surugadai Nihon University Hospital, Tokyo, Japan

To clarify whether the clinical findings of herpes zoster correlate with the vascular changes and localized immune reaction, skin lesions from each clinical stage were examined and compared by immunohistochemistry. Skin biopsies were performed in 20 herpes zoster patients: seven at the erythema stage, eleven at the vesicle stage, and two at the ulcer stage. ICAM-1, CD 4, CD 8 and Substance P in cryosections were stained with their specific antibodies by the ABC method, and paraffin sections were stained with H&E. ICAM-1 was mainly confined to the upper layer of the skin in the erythema stage and to the upper and middle layers in the vesicle stage, while they reached the deeper layer in the ulcer stage. Numerous CD 4 positive and CD 8 positive cells were found around the blood vessels in the deeper layer of the skin as the disease progressed. Substance P was not found by the techniques used. These results suggest that both the vascular lesions and the localized immune reaction do correlate with the clinical stage of the disease in herpes zoster, and that the lesions develop in the upper layer and progress to the deep layer of the skin.

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MORPHOLOGICAL CHANGES OF INTERMEDIATE FILAMENTS IN VIRUS-INFECTED CELLS. Kenji Hiroi, Masahiro Matsunaka, Departmentof Dermatology, WakayamaMedical College, Wakayama, Japan

In virus infection of the skin, epidermic cells show such histopathological features as round shaping and giant cell formation. To examine the internal structure of the cells which suffered morphological changes due to virus infection, we observed possible changes of cytoskeleton, especially intermediate filaments (IFs) having a higher stability. RK13 cells incubated on a coverslip were infected with cowpox virus(CPV) and Herpes virus-1 (HSV1); An indirect immunofluorescence test using MAb against cytokeratin(CK), vimentin(VM), and CPV nucleoprotein(NP) antigen was done. In CPV-infected cells, CK, VM and NP antigen were aggregated in one place of the cytoplasm. In HSV1-infected giant cells, CK was restructured over the whole cell, while VM was ruptured. Differences in changes of IFs due to viral species may be related to the difference in activation of the immunity.

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ESTABLISHMENT OF A T-CELL LINE CONTAINING EPSTEIN-BARR VIRUS GENOME AND ITS CHARCTERISTICS. Hideo Asada, Koji Hashimoto, Natsuko Okada, and Kunihiko Yoshikawa, Department of Dermatology, Osaka University School of Medicine, Osaka, Japan

Epstein-Barr virus (EBV) is generally known to be a human oncogenic B-lymphotrophic herpesvirus. Recently, several reports suggested that EBV was also associated with some cases of T-lymphoproliferative diseases. However, there have been no report on successful culture of EBV genome-positive T lymphocytes from such patients. In the present study, we attempted to establish T-cell line from a patient with EBV genome-positive chronic T-cell leukemia. The leukemic cells have been cultured continuously in RPMI-1640 supplemented with 10% FCS and 40 U/ml IL-2 for more than 18 month and have achieved more than 100 passages. This T-cell line was studied by flow cytometry, May-Grünwald-Giemsa staining, karyotype analysis, Southern blot hybridization, in situ hybridization, and 12-O-tetradecanoylphorbol-13-acetate (TPA) treatment. The established T-cell line was identified as $CD3^+$ $CD4^-$ $CD8^+$ T lymphoblast with many azurophilic granules in its cytoplasm. Clonotypic episomal EBV DNA was detected by Southern blot hybridization with EBV-terminal fragment probe, and EBV-encoded small RNAs (EBER-1 and -2) were demonstrated by in situ hybridization. EBV replication was observed after TPA treatment of this cell line. These results demonstrated the latent infection of EBV in this T-cell line and a probable involvement of EBV in its immortalization mechanism.

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STUDY ON INFECTION OF CYTOMEGALO VIRUS IN SYPHILITIC Xiong chaodong, Huan lihua and Wang haili, Shenzhen Health and Quarantine Bureau, Shenzhen, China

Infection by cytomegalo Virus(CMV) will cause serious consequence. It is important to find out transmiting way of CMV. 87 Syphilitic antibody po -sitive sera and 93 syphilitic antibody negative sera were tested with method of antibody catch ELISA for CMV-IgM.There were 45 CMV-IgM positive sera in syphilitic positive group; the positive rate was 51.7%.There were 28 CVM-IgM positive sera in syphi -litic negative group;the positive rate was 31.1%. There is a very notable discrepancy between syphi -litic and non-syphilitic group. It proves that infectious rate of CMV is much higher in people with sexually transmitted diseases (STD) than in non-STD. The results point out that sexually trans -mission may be a important way of CMV infection.

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EVIDENCE THAT E7 GENES OF SOME CUTANEOUS AND GENITAL HUMAN PAPILLOMAVIRUSES TRANS-ACTIVATE TRANSCRIPTIONAL PROMOTERS E2F-DEPENDENTLY. Atsuro Hiraiwa, Ayumi Adachi and Masaru Ohashi, Department of Dermatology, Nagoya University School of Medicine, Nagoya, Japan

E2F is a transcriptional factor found in many types of mammalian cells, and controls expression of several genes (DNA polymerase alpha, proliferating cell nuclear antigen, dihydrofolate reductase) by binding to E2F sites, a palindromic sequence, on their promoter sequences. In order to elucidate whether E7 genes of human papillomaviruses types 16 (associated with cervical cancer) and 47 (associated with epidermodysplasia verruciformis) could trans-activate expression of these genes indirectly by modulating E2F factor, reporter gene constructs carrying "minimum" promoters so as to produce luciferase or the same reporter constructs added with E2F sites were transfected together with E7 gene-expression vectors into cultured cells, and luciferase activity was assayed. Results indicate that reporter genes added with E2F sites are transcribed several folds strongly than reporter genes without E2F sites. In other words, results indicate that E7 genes of these viruses trans-activate the above described genes indirectly through modulating E2F.

This work has been done in collaboration with Tohru Kiyono and Masahide Ishibashi of Laboratory of Viral Oncology, Aichi Cancer Center.

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Th2 NATURE OF ULTRAVIOLET B-INDUCED SUPPRESSOR T CELLS IN MURINE CONTACT PHOTOSENSITIVITY. Hiroaki Yagi, Yoshiki Tokura and Masahiro Takigawa, Department of Dermatology, Hamamatsu University school of Medicine, Hamamatsu, Japan

Suppression of murine contact photosensitivity (CPS) to 3,3',4',5-tetrachlorosalicylanilide (TCSA) by preirradiation of ultraviolet B (UVB) to the photosensitizing site is caused by antigen-specific, afferent limb-acting, CD4+, suppressor T cells (Ts). Spleen cells from UVB-preirradiated, TCSA-photosensitized mice produced higher levels of IL-4 than controls without higher production of IL-2. These spleen cells were cultured in the presence of rIL-4 and TCSA-photo coupled spleen cells (photoTCSA-SC) as an antigenic stimuli. After several antigenic pulses with photoTCSA-SC, 98.5% of cultured cells had a CD3+,CD4+,CD8- phenotype and these pulsed T cells (Tp) had an in vivo and in vitro suppressive activity in an antigen specific fashion; transfer of Tp to naive recipients rendered them unresponsive to challenge reaction, and the in vitro proliferation of immune lymph node cells (DTH-LNC) was inhibited by the addition of Tp in an afferent limb-acting manner. Thus, Tp represented highly purified antigen-specific Ts concerned with CPS to TCSA. Tp proliferated well to photoTCSA-SC or irradiated DTH-LNC that contained antigen-bearing accessory cells in the presence of rIL-4. Culture supernatants from Tp upon antigenic stimulation showed IL-4 but not IL-2 activity. These results suggested that UVB-induced Ts belong to Th2 in their cytokine profile.

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CLINICAL SUBTYPES AND PHOTOTESTING STUDIES ON 311 CHINESE PATIENTS WITH POLYMORPHOUS LIGHT ERUPTION. Kanghuang Liao, Yi Wang and Yuqing Xia, Institute of Dermatology, Shanghai Medical University, Shanghai, China

The interindividual polymorphism of the lesions and the different action spectra in polymorphous light eruption (PMLE) make the dianose more difficult. 330 patients with suspected PMLE has been studied for 3 to 11 years. Of these, 19 cases including chronic actinic dermatitis (11 cases), summer prurigo (3 cases), and SCLE (1 case) were excluded. Analysis of the remaining 311 cases indicated that the age of onset in most (84.7%) of the patients was 16-40 years, the female to male ration was 4.1:1. Five subtypes were recorded as papulovesicles (118 cases),small papules (103 cases), large papules (35 cases), edematous erythema (26 cases), and mixed rashes (29 cases). Minimal erythema dose (MED) for both UVA and UVB were tested. Bivariate correlation analysis showed a positive correlation between MED-UVA and MED-UVB in normal control ($p<0.05$) but not in PMLE and all its five subtypes. Most PMLE cases had a normal values of MED-UVA and MED-UVB. Lowed MED-UVA ($p<0.05$) and MED-UVB ($p<0.005$) were showed in small papules subtype, and lowed MED-UVB ($p<0.05$) in edematous erythema subtype, indicating the action spectra were not same in all PMLE subtypes. These results show that the term "PMLE" still encompasses several photosensitivity conditions with different pathogenetic mechanisms.

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INTERCELLULAR ADHESION MOLECULES IN ACTINIC PRURIGO.

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Actinic prurigo is a photodermatosis which is most likely an immune mediated disease with lesions appearing late after sun exposure and without a known antigen responsible for this condition. Skin biopsies of patients with actinic prurigo were studied using an immunoperoxidase technique with avidin-biotin, to detect adhesion molecules. Monoclonal antibodies to ICAM-1 (CD54), LFA-1 (CD11a/CD18), CD2 and LFA-3 (CD58) were used as primary antibodies. Skin biopsies from induced actinic prurigo and lesional skin were investigated. An expression of ICAM-1 was detected in epidermis overlying positive areas to ICAM-1 in dermis. This circumstance suggests a similar pathogenesis between actinic prurigo and allergic contact dermatitis. These results are identical to those obtained in polymorphous light eruption,which supports the idea that polymorphous light eruption and actinic prurigo are two different expressions of the same immuno pathological aberration.

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ENHANCED ELASTIN GENE EXPRESSION IN CHRONICALLY PHOTODAMAGED SKIN: Localization of mRNAs by *in situ* Hybridization and Evidence for Transcriptional Activation of the Gene by Transient Transfections with Promoter/Reporter Gene Constructs

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Cutaneous aging, characterized by progressive degenerative changes in the skin, consists of two clinically and biologically distinct processes, (a) the innate (intrinsic) aging and (b) actinic damage on the sun-exposed area of the skin. Histopathologically, sun damaged skin contains "elastotic" material seen in the upper and mid-dermis which stains positively with elastin stains such as Verhoef van Gieson stain. However, the precise biochemical composition of this material is not clear. Previous immunofluorescence staining studies have suggested that the elastotic material consists of in large part elastin. However, the changes in resident fibroblasts responsible for the increased, but non-functional elastin have yet to be illucidated. We have examined the expression of the elastin gene in photodamaged skin in comparison to sun-protected area from the same individuals. The results utilizing Northern analyses of tissue and cultured fibroblasts, *in situ* hybridization and transient transfections with human elastin promoter/reporter gene (CAT) construct indicate upregulation of the elastin gene expression at the sun-exposed area of the photodamaged skin. This upregulation of elastin is maintained in tissue culture. *In situ* hybridization localized the increased elastin mRNA production to the papillary dermis. This corresponds to the site of maximal sun damage. Transient transfections point to increased promoter activity as the possible mechanism for the elevated elastin mRNA expression. These data give insight into the mechanisms of connective tissue alterations seen in photodamage and changes associated with cutaneous aging.

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Influence of Chinese Herb Inchinkoto on the production of Lipid peroxide in porphirin photoxic reaction

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In recent years,there has been much attention on Lipid peroxide in cutaneous diseases,carcinomas and aging. It has been reported that the production of Lipid peroxide(Lpo) is involved in porphrin phototoxic reaction. We have confirmed that Lpo is produced after irradiation by visible Light on hematoporphyrin microsome suspension.

In general,β-carotene is the most popular protective agent for porphyrin phototoxicity. But we can not use β-carotene for porphyrias, becouse it has not been approved by The Ministry of Public Welfare of Japan. Therefor,we considered other carotenoid substances which can be used as phoyoprotecting agents.There are varienty of chinese herbs for used in Japan. Some of these chinese herbs contain β-carotinoid substances. We selected a chinese herb "Inchinkoto" as photoprotecting agent in this study. Inchinkoto showed suppresion on the production of Lipid peroxide.

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APPLICATION OF PHOTODYNAMIC THERAPY IN MYCOSIS FUNGOIDES.

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Photodynamic therapy (PDT) consists of the combination of photosensitizers absorbing light in the red spectral region and irradiation with light of corresponding wavelengths. Here we report in vitro and in vivo studies in mycosis fungoides (MF).

To analyze **in vitro** effects of PDT in comparison to PUVA treatment, cell line MyLa was cultured in the presence of either 8-methoxypsoralen (8-MOP, 10 μg/ml) or the hematoporphyrin derivate photosan (10 μg/ml) for 8 hours followed by a single exposure to UVA (0.5 to 15 J/cm^2) or visible light emitted by a dye laser (630 nm). Whereas both photosensitizers alone did not affect ^{3}H-thymidine uptake, PDT decreased proliferation by 50 % at doses of 1.0 J/cm^2 (=ED_{50}). The ED_{50} of PUVA was 10 fold lower. Video-intensified fluorescence microscopy showed photobleaching of photosan. In contrast, irradiation of 8-MOP resulted in a strong increase of fluorescence throughout the cells.

In vivo fluorescence recordings on plaque lesions of patients with MF treated locally with photosan showed progressive bleaching of the photosensitizer with increasing doses.

Thus, PDT might be a potent alternative to PUVA in the treatment of cutaneous T cell lymphomas, particularly due to its superior tissue penetration.

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COMPARITIVE IN VITRO EFFECTS OF MONO- AND POLYCHROMATIC PHOTODYNAMIC THERAPY ON MALIGNANT TRANSFORMED B- AND T-CELLS.

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In photodynamic therapy (PDT) of precancerous and neoplastic skin diseases recent interest has focused on topical sensitizer application avoiding systemic photosensibilization and on the development of polychromatic light sources, which simultaneously would target different molecular species of a given sensitizer and moreover provide larger irradiation fields as compared to monochromatic dye laser light. In cutaneous lymphomas topical PDT could proof superior to photochemotherapy (PUVA), since its longer wavelength in the red spectral range allows for a deeper light penetration into the irradiated skin. After demonstrating equal antiproliferative efficacy of both, PUVA and PDT on T-cell lymphoma lines we compared inhibitory effects of polychromatic (600-700nm, Waldmann 1200 prototype) and monochromatic (630 nm dye laser) PDT. Malignant transformed T-cell lines (Myla, HuT 78), established from patients with mycosis fungoides and Sézary syndrome, respectively, and Burkitt lymphoma derived B-cell line RA-1 were incubated with different photosensitizers (5-ALA, HpD, methylenblue, $TPPS_4$; T4MPyP; 1, 10, 100 µg/ml) for 2 hours prior to irradiation (fluences 0.5 to 15 J/cm^2). Cellular morphological changes and sensitizer distribution pattern were analyzed before and after light exposure by video-intensified fluorescence microscopy. Individual sensitivity was highest for B-cell line RA-1. However, proliferation as determined by ^{3}H-thymidine uptake was equally inhibited (measured as ED_{50}, equivalent to energy dosage resulting in a 50% inhibition) by both light sources for each single cell line, while sensitizers alone had no antiproliferative efficacy. In conclusion, our present data suggest a potential usefullness of topical polychromatic PDT in cutaneous lymphomas and are in alignment with our preliminary clinical results.

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COMPARISON OF BROADBAND UVA AND (311 nm) UVB IN PHOTOCHEMOTHERAPY OF PSORIASIS.

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The action spectrum for phototoxic erythema peaks around 330 nm and shows the same relative activity at 313 and 365 nm. In three half side comparison studies, using bath water and oral 8-MOP delivery, we compared the therapeutic efficacy of 311nm UVB and broad-band UVA. The irradiation sources were either Philips TL 01 or conventional broad band UVA (Philips TL 09) fluorescent tubes. First, we could demonstrate higher erythemogenic, melanogenic and therapeutic efficacy of the same 311nm dose with oral psoralen in comparison to 311nm UVB radiation alone. Then we compared the two radiation sources after oral 8-MOP administration on the basis of equal erythema. We found UVA slightly more efficient. Comparing 311nm UVB und UVA after bath water sensitization with a 0,001% 8-MOP solution, 90% of the patients showed more rapid clearing on the 311nm exposed side. No patient showed a better result on the UVA treated side. With oral as well as with bath water sensitization we found the cumulative UVA dose about twice as high as with 311nm UVB. Narrowband (311 nm) UVB is efficient with oral and with topical 8-MOP in the treatment of psoriasis.

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APPLICATION OF PHOTODYNAMIC THERPY IN PSORIASIS.

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PUVA is a well established treatment modality for psoriasis. Photodynamic therapy (PDT) consists of the combination of photosensitizers absorbing light in the red spectral region and irradiation with light of corresponding wavelengths. Here we report of in vitro and in vivo studies in psoriasis. We analyzed the **in vitro** effects of these regimens on peripheral mononuclear cells from 6 patients with chronic plaque-stage psoriasis. Cells were incubated with 10 µg/ml of either 8-methoxypsoralen or the hematoporphyrin derivate photosan followed by a single exposure to UVA or visible light emitted by a dye laser (630 nm). IL-6, IL-1ß and TNF-α were measured using IRMA and ELISAs, respectively. PUVA treatment resulted in a decreased production of all three cytokines, particularly IL-6. PDT caused a similar, but less pronounced change in the cytokine pattern: After application of 3 J/cm², IL-6 production decreased by 80% in the case of PUVA, but only by 44% when PDT was used. **In vivo** fluorescence recordings were performed on psoriatic plaques after topical application of photosan. Under irradiation, bleaching of the photosensitizer was observed indicating photochemical reactions. These reactions were strictly limited to the areas pre-treated with the photosensitizer. Thus, PDT might be a therapeutic alternative to PUVA. Its lower effectiveness in vitro might be compensated in vivo particularly due to its superior tissue penetration.

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AN EVALUATION OF PHTHALOCYANINES AS PHOTOSENSITIZER IN PHOTODYNAMIC THERAPY.

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We investigated the spectroscopic properties, the sensitizing efficiency (yield of active oxygen production) in vitro and the photodynamic efficiency of phthalocyanine derivatives (tetrasulfonate=PcS; aluminum PcS=Al-Pc; zink PcS=Zn-Pc) of squamous cell carcinoma in vivo as follows: (1) measurements of the absorption, fluorescence spectra and the fluorescence lifetime in various solutions (buffer, micellar, bovine serum albumin and calf thymus DNA aqueous solutions); (2) determination of the yields of active oxygen produced in the photosensitization by spin trapping method of electron spin resonance (ESR); (3) observation of the growth curve and histological changes of tumor tissue in C3H mice after ruby laser irradiation (80J/cm²) with the phthalocyanines (694.3 nm; 30 µ g/g.b.w).

In the results, (1) the absorbances at long wavelength (675-683 nm) corresponded to the monomer increased in order to PcS<Zn-Pc<Al-Pc and fluorescence intensity increased with the increase of the absorbance at the monomer band. The fluorescence life time corresponded to the monomer was larger than that to the aggregate. (2) The yield of OH: produced in the photosensitization depended on the ratio of the monomer component. (3) The delay of tumor growth was observed in either group of laser treatment with or without photosensitizers but its tendency was remarkable in groups of laser treatment with photosensitizers, especially with Zn-Pc. The necrosis and bleeding more increased in groups of laser treatment with photosensitizers than without photosensitizers.

In conclusion, it is considered that the effective component for photodynamic therapy with the laser light was the monomer of phthalocyanine derivatives, accordingly, the more active sensitizer might be Al-Pc or Zn-Pc which were monomerized easily in various aqueous solutions.

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REGIONAL VARIATION OF PUVA ERYTHEMAL SENSITIVITY.

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The rate of clearance of psoriasis with PUVA varies according to body site, with lesions on the legs clearing slower than those at other sites. In order to determine whether this difference in clearance could be explained by variation in the susceptibility of the skin to psoralen photosensitivity, we have examined the influence of anatomical site on PUVA erythema.

We studied 24 patients about to start PUVA for psoriasis. Two hours after ingestion of 8-methoxypsoralen (0.6 mg/kg), 10 sites (each of 1 cm diameter) on the uninvolved skin of each patient's back, inner forearm and/or thigh were exposed to a geometric series of doses of UVA (dose range 0.35 - 14 J/cm²). The minimal phototoxic dose (MPD) was judged visually at 72 h after irradiation. The intensity of erythema was also measured quantitatively using a reflectance instrument in order to construct dose-response curves for psoralen erythema at each site.

The MPDs measured on the back (median 2 J/cm²) and forearm (median 2 J/cm²) were not significantly different, but the thigh showed significantly reduced erythemal sensitivity (median MPD 8 J/cm²) ($P < 0.01$). The slopes of the dose-response curves were not significantly different at the 3 sites.

The decreased erythemal sensitivity on the leg could explain the slow therapeutic response of psoriasis at this site, where higher doses of UVA should be tolerated without burning. That no difference in sensitivity was found between the arm and the back shows that either of these sites are suitable for routine phototesting of patients to determine the MPD at the start of a course of PUVA.

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THE EFFECTS OF CHELATORS OF DIVALENT CATIONS ON PSORALEN SENSITIZED ERYTHEMA AND HEMOLYSIS.

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PUVA-therapy is accompanied by erythema, hyperpigmentation and other side effects. We found that psoralen photosensitized erythema as well as PUVA-hemolysis was dependent on fluence rate of UV-A radiation. At equal doses of UV-A the degree of reddening of skin was more pronounced after high fluence rate (HFR, 180 W/m²) than after low fluence rate (LFR, 24 W/m²) UV-A irradiation. Chelators of divalent cations (ethylene diamine tetraacetic acid, desferrioxamine and o-phenanthroline) applied on human skin two hours before PUVA-treatment influenced erythema. Chelators decreased the degree of reddening 1.5-2 times in the case of LFR PUVA-erythema, and enhanced reddening in the case of HFR PUVA-erythema. Similar effects of chelators were found in PUVA-hemolysis induced at LFR and HFR conditions of irradiation. Thus PUVA-hemolysis is a convenient model for investigation of mechanism of photodynamic effects of psoralen in human skin. It is proposed that in both cases inhibition of effects of LFR PUVA-treatment occurs due to chelating of Fe^{2+} ions, while enhancement of HFR PUVA-treatment effects may be due to chelating of Ca^{2+} ions.

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***IN VIVO* STUDY OF PROTECTIVE EFFECT BY HEAT AND COLD TREATMENTS AGAINST UVB INJURY.**

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To understand the interaction between UVB damage and environmental temperature, the UVB-mediated sunburn cell (SBC) formation in the skin of the mice treated with heat and cold applications was examined.

To the outer surface of the ear of BALB/c mice, the metal plate was attached in the constant-temperature bath maintained at 42 °C and in an ice bath at 0 °C for 20 min at various intervals (0h, 6h, 12h, 24h) prior to UVB treatment (100 and 300 mJ/cm²). The number of SBC of the ear skin was counted by routine techniques to quantify epidermal damage by UVB. Heat treatment showed depletion of SBC formation in each UVB dose. Cold application significantly decreased the number of SBC. The result in the mice exposed to heat stress supports the previous report that demonstrated the suppression of SBC production by preliminary treatment of infrared radiation (Danno et al., 1992). In addition, our present study showed that early heat treatment was also efficient in the depletion. It appears that significant reduction of UVB injury by cold stress has never been reported. Inducible molecules in the epidermis by those loads, including heat shock proteins and metallothionein, should be studied, because there is no persuative explanation for the reduction of UV damage.

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TRANSCRIPTIONAL ACTIVATION OF THE C-JUN PROTOONCOGENE - BUT NO INDUCTION OF AP-1 DNA-BINDING BY PHOTODYNAMIC THERAPY.

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Photodynamic therapy (PDT) is currently under investigation for the treatment of tumors with superficial localization, especially practicable for the skin. This novel therapeutical approach is performed by the topical or systemic application of a photosensitizer and selective activation by illumination with tissue penetrating visible light from lasers or non-coherent light sources. Clinical studies, so far, have demonstrated efficacy and safety in the eradication of early stage tumors as well as in palliation. Despite of these promising results, the molecular basis of cell death and inflammatory reaction following PDT is still unknown. In this study, we analyzed the mechanisms and kinetics of gene activation, relevant for the human immune response and the cytotoxic effects of photodynamic treatment. Human cell lines (Jurkat/Mono Mac 6/A431) were preincubated for one hour in various concentrations of the hematoporphyrinderivate PhotofrinR, commonly used in clinical PDT. The subsequent illumination of the cells was performed by an argon pumped dye laser with a power density of 40 mW/cm² for 100 s at a wavelength of 630 nm. Total cellular RNA was isolated and blots were sequentially hybridized with probes specific for early response genes which are activated also upon UV and gamma radiation. Nuclear extracts were prepared for electrophoretic mobility shift assays (EMSA) to demonstrate the functional DNA binding of various transcription factor systems. In the Jurkat cell line photodynamic treatment induced c-jun mRNA levels in a dose dependent and transient fashion with a maximum rate at 2 h and 4 h. Electrophoretic mobility shift assays showed no increase of the binding activity of AP-1 to the corresponding element at 0.5 h to 8 h at various sublethal and cytotoxic doses. In addition, no increase in DNA binding activity was observed for the nuclear factors CREB, NF-jun and NF-kB. In conclusion, these results point at a divergent molecular mechanism for PDT, compared to UV and gamma-ray treatment. Because the nuclear factors AP-1, NF-jun and NF-kB are not activated by PDT, we further search for the responsible mechanisms underlying c-jun induction.

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CLOTHING PREVENTS ULTRAVIOLET B RADIATION DEPENDENT PHOTOSYNTHESIS OF VITAMIN D3. Lois Matsuoka, Jacobo Wortsman, Michael Dannenberg, Bruce Hollis, Zhiren Lu, Michael Holick, Jefferson Medical College, Philadelphia, PA; Southern Illinois School of Medicine, Springfield, IL; Medical U. of South Carolina, Charleston SC; Boston U. School of Medicine, Boston, MA, USA

Photoprotection of skin is mainly a function of clothing although its effectiveness against ultraviolet light B solar radiation (wavelengths 290-320 nm; UVB) has not been measured in vivo. Since UVB mediates the cutaneous formation of vitamin D3, we examined the attenuation of that photosynthetic reaction by cotton, wool and polyester fabrics, in black and white colors. Direct transmission of UVB was attenuated the most by black wool (98.6% of incident irradiance), and the least by white cotton (47.7%). No fabric allowed the photoproduction of previtamin D3 from 7-dehydrocholesterol irradiated in vitro with up to forty minutes of simulated sunlight, or the elevation of serum vitamin D3 after irradiation with approximately one minimal erythema dose (MED) of UVB in volunteers wearing jogging garments made of these fabrics. Increasing the whole body irradiation dose to six MEDs still failed to produce a serum D3 response in garment-clad subjects. Regular (seasonal) street clothing also prevented an elevation of serum D3 in response to UVB irradiation. Clothing prevents or significantly impairs the formation of vitamin D3 after photostimulation with up to six MEDs of UVB.

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EFFECTIVENESS OF A COMBINATION OF VITAMINS C AND E IN INHIBITING UV DAMAGE TO PORCINE SKIN. Douglas Darr, Stan Dunston*, H. Kamino, H. Faust and Sheldon Pinnell, Div. of Dermatology, Duke Univ. Medical Center, Durham, N.C., *North Carolina State Univ. School of Vet. Medicine, Raleigh, N.C.

The treatment of various dermatologic disorders using topically applied vitamins has become an area of increasing interest. Of particular interest has been the use of anti-oxidant vitamins such as Vitamin C (VC), Vitamin E (VE), and β-carotene to protect against oxidative damage, e.g. in solar damage and inflammation. It has been long known that, particularly in the case of VC & VE, these anti-oxidants work in concert to inhibit cellular damage. However, we have not noted any studies systematically investigating the effects of combining antioxidants for topical use.

In this study, we assessed the ability of VC, VE or the combination to inhibit UVB or PUVA damage to porcine skin when topically applied. Using erythema and "sunburn cell" (SBC) formation as endpoints, we noted that VC protection is maximum at 5%-10% (w/v). Vitamin E inhibited erythema with increasing concentration (up to 3% w/v) consistent with its ability to "screen" some of the UVB rays as well as act as an antioxidant.The combination of the two vitamins provided additive protection. Interestingly, inhibition of SBC formation ($\approx$80% for a 2-3 MED Dose) appears to reach a plateau at approximately 1% VE concentration in the formulation.

In PUVA studies, a blinded histopathological analysis was employed as SBC quantitation was impossible at higher levels of damage. VC again proved quite effective at lessening photo-toxic damage. VE was minimally effective at the levels tested (0.5% -3% w/v). Indeed the higher dosage of VE appeared the least protective. The combination of VC and VE (at 2% w/v) was slightly better at PUVA protection than VC alone. Thus, this study shows that by combining Vitamin C and Vitamin E in a topical formulation one can maximize UVB and photo-toxic UVA protection in an animal model.

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PHOTOSENSITIZATION OF HUMAN FIBROBLASTS WITH WATER-SOLUBLE AND WATER-INSOLUBLE VITAMINS. Tomoko Maeda, Hiroyasu Taguchi, Kenji Sato and Kunihiko Yoshikawa, Department of Dermatology, Osaka University School of Medicine, Osaka, Japan

Riboflavin and pyridoxine have been known to cause cytotoxic effect after UVA radiation. There are some other endogenous chemicals such as water-soluble and water-insoluble vitamins which have absorption in the UVA range. We studied whether the vitamins produced cytotoxicity after UVA radiation. The measurement of the cytotoxicity was done by using post UVA colony formation of human fibroblasts. The cells in PBS(+) containing test substances were irradiated with UVA from the buttom of dishes. Pyridoxamine showed cytotoxicity after UVA radiation but neither pyridoxal or pyridoxal phosphate showed it. Vitamin K, a water-insoluble vitamins, showed a little cytotoxicity. These photosensitizing vitamins together with riboflavin and pyridoxine may contribute to the photoaging of human skin.

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ULTRAVIOLET RADIATION CAN ALTER THE EXPRESSION OF ICAM-1 AND ELAM-1 ON THE CULTURED ENDOTHELIAL CELL. Mitsuo Yamawaki, Shozo Futamura, Takeshi Horio and Yasuo Asada Department of Dermatology, Kansai Medical University Osaka, Japan

Vascular endothelial cells(EC) play an important role in the emigration from the blood of the leukocytes that participate in the cutaneous inflammatory responses. Ultraviolet radiation (UVR) regulates inflammatory and immunological reactions in the skin. We examined the effect of UVR on the expression of ICAM-1 and ELAM-1 induced by TNF-α on the cultured human umbilical EC using immunohistochemical techniques. A single exposure to UVB(25 or 50mJ/sq cm) inhibited both ICAM-1 and ELAM-1 induction by TNF-α (10ng/ml) given 10 min before irradiation. However, 10mJ/sq cm of UVB did not inhibited ELAM-1 expression, while ICAM-1 expression was suppressed in the same condition. The UVB exposure at a dose of 25mJ/sq cm 10 min to 24hr before TNF-α treatment also inhibited ELAM-1 but not ICAM-1 expression. Pre-irradiation of UVB at a dose of 10mJ/sq cm inhibited neither adhesion molecules. However, 10mJ/sq cm of UVB elongated the strong expression of ELAM-1 by TNF-α until 12hr, while TNF-α alone induced a peak at 4hr and much weaker expression at 12hr. These results suggest that UVR may affect cutaneous inflammatory reactions via its effect on the expression of adhesion molecules of EC.

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MELANIN REDUCES ULTRAVIOLET-INDUCED DNA DAMAGE FORMATION AND CELL KILLING RATE IN CULTURED HUMAN MELANOMA CELLS. Nobuhiko Kobayashi, Tsutomu Muramatsu, Yukio Yamashina, Masami Yamaji, Haruhiko Ohno, Toshihiko Shirai, *Ron Hashizume and **Toshio Mori, Department of Dermatology and **RI Center, Nara Medical University, Kashihara, and *Institute for Advanced Skin Research INC., Tsukuba, Japan

Intracellular melanin is considered to protect the underlying cell nucleus against ultraviolet(UV)-induced DNA damage formation, although related published data have been confusing. We previously reported that melanin reduced the formation of both cyclobutane pyrimidine dimers and (6-4)photoproducts, and reduced the cell killing rate as well, using three cultured human melanoma cell lines with different pigment levels. The three cell lines, however, might have different genetic backgrounds (possibly, different UV sensitivities and different induction rates).

The purpose of the present study was to determine the protective effect of melanin itself in two types of melanoma cells with the same genetic background, irradiated with UV-B and UV-C light. We prepared less melanotic cells from highly melanotic melanoma cells by treating with docosahexaenoic acid (DHA). Two types of DNA damage were determined by ELISA with monoclonal antibodies specific for these photolesions. We found that DHA treated less melanotic cells formed more UV-induced (6-4)photoproducts than highly melanotic control cells. The determination of cyclobutane dimers is in progress. We also found that less melanotic cells were more UV sensitive than control cells. These results suggest that intracellular melanin reduces UV-induced DNA damage formation and cell killing rate.

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CONTRIBUTION OF ACTIVE OXYGEN SPECIES TO PYRIDOXINE PHOTOSENSITIZATION. Hiroyasu Taguchi, Tomoko Maeda, Kenji Sato and Kunihiko Yoshikawa, Department of Dermatology, Osaka University School of Medicine, Osaka, Japan

Recently, we demonstrated that UVA-irradiated pyridoxine had cytotoxic effect on cultured human fibroblasts and that the effect remained for at least 60 min after radiation. The possible cause of the cytotoxicity is photodegradation product(s) of pyridoxine and/or active oxygen species such as hydrogen peroxide. In this study, we examind whether active oxygen species contributes to pyridoxine photosensitization. The cytotoxicity of pyridoxine after UVA radiation decreased when it was irradiated under anaerobic conditions. Hydrogen peroxide was produced by UVA radiation, but its amount was too little to cause cell killing. This was consistent with the finding that there was no recovery of survival when the cells were irradiated with UVA in the presence of both pyridoxine and catalase. These results indicate that at least hydrogen peroxide is not involved in pyridoxine photosensitization.

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ENHANCED EXPRESSION OF HEAT SHOCK PROTEINS IN KERATINOCYTES IRRADIATED WITH UV LIGHT. Hideyuki Ichikawa, Tsuzuru Takura, Toshie Osaka, Yoshihisa Sato, Shoji Fukushima, Institute for Advanced Skin Research Inc., Yokohama, Japan

We previously reported animal models of autoimmune like persistent photosensitivity in guinea pigs. Influences of ultraviolet irradiation on the induction of heat shock proteins(HSP) was investigated to clarify the relationship between HSP and persistent photosensitivity. Monoclonal anti HSP60, 72 and 90 antibodies were used to detect HSP in human and murine keratinocytes after irradiation of UVA or UVB.

Expression of both HSP 72 and 90 were enhanced after irradiation of UV lights. HSP 72 was localized in nucleus in the early stage after UV irradiation, and also in cytoplasm in the later stage. Augmented expression of HSP 90 was observed mainly in cytoplasm. However, significant increase in expression of HSP 60 was not observed.

These results suggest that HSP induced by UV irradiation plays an important role in the response of photosensitivity.

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FACTORS INVOLVED IN THE SYSTEMIC IMMUNOSUPPRESSION BY UVB RADIATION. Hiroko Miyauchi, Takeshi Horio and Yasuo Asada, Department of Dermatology, Kansai Medical University, Moriguchi, Japan

Irradiation of mice with UVB can suppress contact hypersensitivity(CHS) "systemically", even if the hapten is applied to the non-irradiated site. There is no standarized model to induce this systemic immunosuppression. We investigated the effect of following factors on DNFB-CHS; UVB-dose, divided exposure, timing of sensitization after irradiation, area of exposure, hapten concentration, age, and genetic basis. The suppression was enhanced by increasing UVB dose. When 1J/sq cm of UVB was exposed, divided daily exposure(0.5Jx2, 0.25Jx4) was more suppressive than single exposure. 5 days interval between irradiation and sensitization induced stronger suppression than 1 or 2 days interval. The suppression was detected even by sensitization 10 days after or 1 day before exposure. When the total energy(J) was kept constant, the exposure of low dose-UVB to the large area(0.5J/sq cm x16sq cm) suppressed CHS more strongly than high dose-UVB to the small area(2J/sq cm x4sq cm). When 25ul of DNFB-solution was applied, higher concentration induced lower suppression. The stronger suppression was caused in the young(7w) than in the old(32w) mice. These results suggest that not only UVB dose but also various other factors should be taken into consideration to induce effectively the systemic immunosuppression.

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ROLE OF HYDROGEN PEROXIDE IN UVA-INDUCED RIBOFLAVIN CYTOTOXICITY. Kenji Sato, Hiroyasu Taguchi, Tomoko Maeda and Kunihiko Yoshikawa, Department of Dermatology, Osaka University School of Medicine, Osaka, Japan

Riboflavin-containing medium exposed to UVA caused a marked cytotoxic effect when placed on human fibroblasts. The cytotoxic materials produced were stable for at least 40 min after irradiation. When riboflavin-containing solutions were irradiated with UVA, the riboflavin spectrum was degraded in a dose-dependent manner. The cytotoxicity was mostly caused by hydrogen peroxide produced in the irradiated riboflavin solution because the solution lost hydrogen peroxide by catalase treatment and the resultant restoration of survival was almost complete. One molecule of hydrogen peroxide was calculated to produce by two molecules of riboflavin after UVA radiation. Hydrogen peroxide produced from irradiated riboflavin may be one of the causes for photoaging of human skin.

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DOSE-RESPONSE EFFECTS OF ACUTE ULTRAVIOLET IRRADIATION ON ANTIOXIDANTS AND MOLECULAR MARKERS OF OXIDATION IN MURINE EPIDERMIS AND DERMIS. Yasuko Shindo, Eric WITT, Derick Han and Lester PACKER, Department of Molecular and Cell Biology, 251 Life Sciences Addition, University of California, Berkeley, USA

In a previous study we examined the antioxidant defense system of the epidermis and dermis. We found;1) Most antioxidants(enzymic and non-enzymic) are present in higher concentrations in epidermis than dermis in hairless mice. 2) When mice were exposed to a single large dose of simulated solar light in vivo the concentrations of many antioxidants decreased dramatically in both epidermis and dermis.

In the present study we tested a range of doses of UV light commomly or occasionally encountered to elucidate the patterns of antioxidant response and the cellular damage over such a range. Hairless mice were irradiated with simulated solar light at doses of 2,5,12.5, and 25Joule/cm^2 and enzymic and non-enzymic antioxidants as well as lipid hydroperoxides were measured in both epidermis and dermis.

Among the non-enzymic antioxidants, two distinctly different dose response patterns were seen. Ascorbate was rapidly depleted at doses between 0-5J/cm^2 but was less affected between 5-25 J/cm^2. In contrast, glutathione, ubiquinol/one, and α-tocopherol levels remained approximately equal to control levels between 0-5J/cm^2, then decreased to varying degrees from 5-25J/cm^2; ubiquinol was almost completely depleted while α-tocopherol dropped only 30 %. The concentration of lipid hydroperoxides increased throughout the dose range. These results may be explained partly by direct destruction of some antioxidants by UV light, partly by the separate antioxidant functions of the compounds, and partly by recycling of some antioxidants at the expense of others.

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CHRONIC ULTRAVIOLET RADIATION-INDUCED CHANGES IN THE SKIN GLYCOSAMINOGLYCANS OF HAIRLESS MICE. Yumiko Nonaka, Katsuyuki Okada, Kazunori Ohnishi, Osamu Ishikawa and Yoshiki Miyachi, Department of Dermatology, Gunma University School of Medicine, Maebashi, Japan

Changes in the skin glycosaminoglycans (GAGs) induced by chronic ultraviolet (UV-A, UV-B) radiation were investigated using hairless mice. Female mice (aged 6 weeks) were irradiated three times a week with UV-A (30 J/cm^2) or UV-B (40 mJ/cm^2). The UV-A or UV-B irradiated and age-matched unirradiated mice were sacrificed after 6 and 12 weeks, and dorsal trunk skins were excised. After removal of the subcutaneous tissue the skin was minced into pieces, defatted with acetone, dried and weighed. To isolate crude GAGs, the skin was treated with 2 % NaOH, neutralized with HCl, digested with pronase, deproteinized with 10 % trichloroacetic acid and dialyzed. Crude GAGs were precipitated with 0.1 % cetylpyridinium chloride, washed with ethanol and dried. After digestion with chondroitinase-AC or chondroitinase-ABC, GAG-derived unsaturated disaccharides were analyzed using precolumn labeling with 1-phenyl-3-methyl-5-pyrasolone by high-performance liquid chromatography. We found contents of main disaccharides (hyaluronic acid-derived ΔDi-HA and dermatan sulfate-derived ΔDi-4S) tended to increase after UV-B exposures (131 % and 117 % of the control mice at 12 weeks respectively) and decrease after UV-A exposures (84 % and 80 %). These results suggest that chronic radiation of UV-A or UV-B has different effects on the GAGs metabolism of hairless mice skin .

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DOSE- AND TIME-DEPENDENT EFFECT OF ACUTE UVB IRRADIATION ON SUPEROXIDE DISMUTASE ACTIVITY IN HAIRLESS MOUSE SKIN. Katsuyuki Okada, Yumiko Nonaka, Osamu Ishikawa, Kazunori Ohnishi and Yoshiki Miyachi, Department of Dermatology, Gunma University School of Medicine, Maebashi, Japan

Effect of acute UVB irradiation on superoxide dismutase (SOD) activity in the skin was investigated using hairless mice. Female mice (aged 6 weeks) were irradiated with UVB at doses of 40, 200 and 1000 mJ/cm^2, respectively at a time. The mice were sacrificed 1, 3 and 7 days after irradiation. Age matched unirradiated mice served as controls. The dorsal trunk skin was excised and stored in liquid nitrogen until examination. SOD activities were assayed spectrophotometrically by cytochrome c reduction method. The SOD activities on the 1st day after irradiation at the dose of 40 mJ/cm^2 (18.6±3.9 U/mg protein; mean±SD, $p < 0.001$) and at the dose of 200 mJ/cm^2 (12.8±2.0, $p < 0.02$) significantly increased when compared with the controls (9.2±1.9), while at the dose of 1000 mJ/cm^2 (6.9±1.5) decreased SOD activities were noticed. The SOD activities at the doses of both 40 and 200 mJ/cm^2 on the 3rd and 7th day after irradiation returned to the level of controls, though those at the dose of 1000 mJ/cm^2 gradually decreased. These results indicate that the increased SOD activities induced by UVB irradiation at relatively low doses decreased in a few days and that UVB irradiation at a high dose does not increase those activities but impair the antioxidant mechanisms.

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THE DIFFERENT EFFECTS OF SUNSCREENS ON UVB ERYTHEMA AND LANGERHANS CELLS DEPRESSION. Tsuguna Miyagi, Abdul Manan Bhutto and Shigeo Nonaka, Department of Dermatology, Faculty of Medicine, University of Ryukyus, Okinawa, Japan

It is a well known fact that UVB supresses Langerhans cell(LC) number and function. Sunscreens are widely used to supress UVB erythema. But there is very little studies on the effects of the sunscreens on the LC. In this study, we investigate sunscreen effects on LC of the epidermis. dd-Y strain mice were used for this study. The UVB light was irradiated to the skin of back of the mice using a SE-30 fluorescence lamp. Two types of sunscreen agents (15 and 28 SPF) were used for this study.

A 100 mJ/cm^2 of UVB induced a marked decrease of LC in the mice. There was no change of LC number when the 15 and 28 SPF of sunscreens were used. However, the size of LC decreasesd in sunscreen group, compared with those in control mice. There was no difference between 15 and 28 SPF of sunscreens. From these results, it appears that sunscreen supresses the UVB erythema and LC size, but not the number of LC. This means that the possibility exists taht subscreens do not protect against UVB supression of LC function. Further studies will be needed to investigate the effect of sunscreens using many more other indicators, including erythema.

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PHOTOTOXIC POTENTIAL OF NEW QUINOLONE ANTIBACTERIAL AGENTS IN RABBITS. Shoshi Yasuda and Takuo Tsuji, Department of Dermatology, Nagoya City University Medical School, Nagoya, Japan

As new quinolone antibacterial agents become widely used, the numbers of reports of photosensitivity reactions to these drugs have been increased. The purpose of this study is to examine the phototoxic potentials of several new quinolone antibacterial agents using rabbits, considering the mechanism of the photosensitivity.

Six kinds of drugs, lomefloxacin (LFLX), enoxacin (ENX), ofloxacin (OFLX), ciprofloxacin (CPFX), tosufloxacin (TFLX) and norfloxacin (NFLX), were examined in this study. Five Japanese white rabbits were used for each drug. The shaved abdominal skin of the rabbit was irradiated with UVA, 1.2 J/cm^2 to 21 J/cm^2 with 50% increments, 1 h after oral administration of drugs. The light source used was Toshiba FL32S·BL equipped with window glass. Minimal erythema doses were determined 48 h after irradiation. Dosage of each drug required for producing erythema by irradiating 9 J/cm^2 UVA (DOSE 9J, mg/kg) was calculated from dose-response curves.

The DOSE 9J values of LFLX, ENX, OFLX, CPFX and NFLX were 46.6, 128.6, 288.5, 441.7 and 640 mg/kg, respectively. This order was relevant to the incidence of photosensitivity reactions clinically observed. No phototoxic reactions were observed with TFLX even when maximal dose (at least 640 mg/kg) was given.

Among these new quinolone antibacterial agents LFLX showed the highest phototoxic potency and it is suggested to be most carefully used for men.

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STUDY ON THE TIME NEEDED TO REMOVE UVB-INDUCED CYCLOBUTANE-TYPE THYMINE DIMERS ON NUCLEAR DNA OF DERMATOPHYTES. Makoto Hori, Kizo Honma, Masao Yamada, Hikotaro Yoshida, Department of Dermatology, Nagasaki University School of Medicine, Nagasaki, Japan

Cyclobutane-type thymine dimers are known to be major photoproducts and induce cell death, when it is not removed. However, UV-light irradiation to dermatophyte is not so effective for the cell death. We examined the time needed to repair UVB-induced cyclobutane-type thymine dimers on nuclear DNA of dermatophytes. Each of six strains of dermatophyte was cultured on 8 dishes of Sabouraud's dextrose agar plate respectively. Thirty-six of 48 samples cultured were exposed to UVB at doses of 700mJ/cm^2. DNA from 36 samples (6 strains) were extracted before, immediately after, 4 hours, 24 hours, 2 days and 4 days after irradiation. Four micrograms of each DNA specimen from each strain were dotted on a nitrocellulose filter at time course, and immunoblotted using monoclonal antibody against cyclobutane-type thymine dimer.

The intensities (%) of the immunoprecipitates were measured using a photo densitometer. Six strains were irradiated with 2,100mJ/cm^2 UVB and compared macroscopically with unirradiated samples. The results indicate that all the 6 DNA extracted immediately after irradiation showed strongest positive reaction. Four DNA specimens from the 6 strains at 4 hours, and 2 DNA specimens at 24 hours after irradiation became negative. The growths of all dermatophytes were not suppressed. These findings suggested that the dermatophytes repair more quickly UVB-induced thymine dimers on their DNA than human epidermal cells.

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COMPARATIVE STUDY OF SKIN SURFACE MICROFLORA IN ATOPIC PATIENTS AND HEALTHY SUBJECTS. T.Ogawa, K.Katsuoka*and S.Nishiyama**, Cosmetics Lab. Kanebo.Ltd.*Yokohama National Hosp.**Dept of Dermatol.Kitasato Univ.Japan

Areas of active dermatitis in patients with atopic dermatitis are known to be heavily colonized with Staphylococcus aureus. However, the degree of colonization by staphylococci (staph) in normal areas or in areas of slight dermatitis is unknown. In order to clarify the difference in bacterial flora between patients with atopic dermatitis and healthy subjects, bacteria were sampled from dermatitic faces and from normal or mildly dermatitic arms of 23 patients by a contact-plate sampling technique. Sampling was similarly performed from faces and arms of 20 healthy subjects as a control. Staph were selectively sampled using mannitol salt medium, and species were determined on the basis of the API-Staph-System and coagulase test. Personal variation in composition and density of staph species in patients and healthy subjects was noted. The density of S.aureus was different between patients and healthy subjects. From faces of patients with severe dermatitis more than 10^5 colony forming units(cfu) per 10cm^2 were counted. Large numbers of S.epidermidis were detected in faces of healthy subjects. On arms of patients, the mean density of S.aureus was 13 cfu/10cm^2, compared to 0.5 cfu/10cm^2 on arms of healthy subjects($P<0.05$). The mean density of the total aerobic flora was 55 cfu/10cm^2 on arms of patients, compared to 21 cfu/10cm^2 on arms of healthy subjects. Several species of coagulase negative staph were detected in both patients and healthy subjects. The contact-plate sampling technique made it possible to count even very low density of bacteria on arms. The number of S.aureus was significantly different between practically normal arms of patients and healthy subjects, although the number of total aerobic flora was not. Our results support the idea that S.aureus is an exacerbating factor in atopic dermatitis.

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CATARACT IN ATOPIC DERMATITIS WITH FACIAL INVOLVEMENT. Shinya Hirano, Norito Katoh, Saburo Kishimoto, Hirokazu Yasuno, Hitoshi Ikebe*, Yoshio Akagi*, Department of Dermatology and Ophthalmology*, Kyoto Prefectural University of Medicine, Kyoto, Japan

Cataract and retinal detachment are important ocular complications in adolescent and adult patients with atopic dermatitis (AD), and atopic cataract has been reported to occur frequently in the more severe patients with facial involvement. Our purpose was to study the factors relevant to cataract formation. So far 112 AD patients with facial involvement were referred to the Department of Ophthalmology, and clinical data of patients with atopic cataract were compared with those of patients without cataract. Serum levels of eosinophil cationic protein (ECP), major basic protein (MBP) and blood eosinophil counts at the time of exacerbated facial dermatitis were also measured. Atopic cataract was observed in 23% of all patients, and the levels of MBP and eosinophils were significantly higher in the patients with cataract than in the patients without cataract. There was no significant difference in the facial dermatitis score, which correlated significantly with ECP and total extent of AD, between the patients with cataract and those without, but the duration of facial dermatitis and exacerbated facial dermatitis was significantly longer in the patients with cataract. Retinal detachment or break was present in nine of 112 patients, but no significant differences in clinical and laboratory data were found between the patients with the retinal disorders and those without. The development of atopic cataract is suggested to be associated with the factors responsible for deterioration of facial dermatitis.

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INFLUENCES OF RESPIRATORY ATOPY ON DISTRIBUTION OF EG2 POSITIVE ACTIVATED EOSINOPHILS IN SKIN LESION OF ATOPIC DERMATITIS.

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Although various authors have stated that infiltration of activated eosinophils plays an important role in the pathogenesis of atopic dermatitis(AD), activated eosinophils are not always observed in skin lesions of AD. To see what the factors might be that are related to the infiltration of activated eosinophils in some AD skin lesions, we took biopsy specimens from both scratched and unscratched lesions of 44 consecutive patients with AD. The specimens were stained with monoclonal anti-eosinophil cationic protein anti bodies, EG1 and EG2. Activated eosinophils were rarely observed in unscratched skin lesions, but were often seen in scratched lesions. The scratched lesions of AD patients with personal history of respiratory atopy showed greater number of activated eosinophils compared to the scratched skin lesions of AD patients who did not have both personal and family history of respiratory atopy. These findings suggeste that the increased number of activated eosinophils is characterstic of scratched skin lesions of AD patients who have predisposition to respiratory atopy.

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PHENOTYPING IN ATOPIC NUCLEAR ADULT FAMILY MEMBERS: EVALUATION OF EACH DIAGNOSTIC PARAMETER FOR ATOPIC DERMATITIS. Tomoe Kawamoto[1], Takashi Yoshiike[1], Yosuke Aikawa[1], Hajime Suto[1], Motomi Miyazaki[2], and Hideoki Ogawa[1], Department of Dermatology, Juntendo University School of Medicine[1], Teijin Bio Laboratories[2], Tokyo, Japan

Although genetic predisposition has been reported in an extended number of atopic families, parameters for evaluation have been limited due to the large number of cases involved in many of studies. In order to explore the genetic background of atopic dermatitis, a number of clinical parameters were carefully examined in atopic family members.

Parents and siblings of adolescent and adult patients with atopic dermatitis in 6 families (24 total members) were studied utilizing Hanifin & Rajka's diagnostic criteria and specific serum IgE to major allergens. Following careful examination, the 54% and 20% out of 4 major and 23 minor diagnostic criteria were respectively satisfied in parents and siblings of patients with atopic dermatitis. According to Hanifin & Rajka's criteria, 38% of the sample group were diagnosed as atopic dermatitis. Total serum IgE was elevated in 28% of the family members of patients, while specific IgE to house dust mite antigens was noted in 38%. Higher frequencies were observed in xerosis/ichthyosis/palmar hyperlinearity/pilar keratosis (100%), itching (56%), chronically relapsing dermatitis (39%) and intolerance to wools/detergents (39%). There were not any instances of Dennie-Morgan's infraorbital fissures, eye involvements, orbital darkening, cervical fissures or facial pityriasis alba.

Although this study is preliminary because of the small sample size and lack of normal controls, it is likely, however, that atopic skin, i.e. dry and irritable skin, is a genetic predisposition of atopic dermatitis.

Additional results on genetic linkage to a polymorphic marker on chromosome 11q13 will be reported.

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LEUKOTRIENE A_4 HYDROLASE IN PERIPHERAL LEUKOCYTES AND SKIN OF PATIENTS WITH ATOPIC DERMATITIS. Hiroko Okano-Mitani, Yuji Horiguchi, Kouichi Ikai and Sadao Imamura, Department of Dermatology, Kyoto University Faculty of Medicine, Kyoto, Japan

The biochemical events underlying inflammatory changes in atopic dermatitis (AD) remain unknown. Leukotriene (LT) B_4, the 5-lipoxygenase product of arachidonic acid possesses various biological activities such as leukocyte chemotaxis and smooth muscle contraction, which can lead to cutaneous inflammation. LTA_4 hydrolase, which catalyzes the conversion of LTA_4 to LTB_4, is a rate-limiting enzyme for LTB_4 biosynthesis. We measured the enzyme activity of LTA_4 hydrolase in the 100,000 x g supernatant of peripheral polymorphonuclear leukocytes (PMN) of patients with AD using HPLC. Based on the extent of dermatitis, AD patients were classified into three groups; "severe" (>50%), "moderate" (50-10%) and "mild" (<10%). Severe AD patients showed higher LTA_4 hydrolase activities (74.38 ± 26.89 pmol/10^6 cells/min, n=5) compared to moderate (18.82 ± 14.56 pmol/10^6 cells/min, n=9) and mild (7.19 ± 5.38 pmol/10^6 cells/min, n=5) AD patients and normal controls (22.98 ± 15.98 pmol/10^6 cells/min, n=12). In these cases, no correlation was found between LTA_4 hydrolase activity and serum IgE levels. We also examined the localization of LTA_4 hydrolase in skin lesions of AD by immunohistochemistry using antibody against human LTA_4 hydrolase. LTA_4 hydrolase was detected in the cytoplasm of epidermal cells and fibroblasts in the dermis, but there was no difference in the immunoreactivity of LTA_4 hydrolase between involved and uninvolved epidermis. These results suggest that LTA_4 hydrolase plays a significant role in at least part of the pathogenesis of AD.

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SEVERITY SCORING OF ATOPIC DERMATITIS: THE SCORAD INDEX European Task Force on Atopic Dermatitis

(J.F. Stalder, A. Taieb, D. Vieluf, B. Kunz, J. Ring, D.J. Atherton, T. Bieber, E. Bonifazi, A. Broberg, A. Calza, R. Coleman, Y. de Prost, T.L. Diepgen, C. Gelmetti, A. Gianetti, J. Harper, J.M. Lachapelle, T. Langeland, R. Lever, A.P. Oranje, M. Poncet, C. Queille-Roussel, J. Revuz, J.C. Roujeau, J.H. Saurat, M. Song, D. Tennstedt, D. Van Neste)

The assessment methods of atopic dermatitis (AD) are not standardized and therapeutic studies are often difficult to interpret. Efforts have been done recently to classify patients in groups of severity and to establish simple and reliable assessment methods. The aims of the European Task Force on Atopic Dermatitis were to provide a tool to record data precisely and consistently for routine evaluations and clinical studies at various institutions and to develop a composite scoring index for AD. Consensus definitions were given for six intensity items: (1) erythema, (2) edema/papulation, (3) oozing/crusts, (4) excoriations, (5) lichenification and (6) dryness. Each item is graded from 0-3 (0=absent, 1=mild, 2=moderate, 3=severe) according to reference photographs. The area chosen for grading must be representative (average intensity) for each item in a given patient as determined by the investigator, thus excluding one 'target' area or the worst affected site. Dryness must be appreciated on areas not involved by acute lesions or lichenifications. The SCORAD (score of AD) combines the grading of the extent (using the rule of nine for body surface involved with regard to the age of the patient), severity/intensity of the skin lesions and subjective symptoms (average value of pruritus and sleep loss for the last 3 days/nights indicated on a 10 cm visual analog scale by the patient). The SCORAD is simple and easy to use in outpatient clinics. Based on mathematical appreciation of weights of the items used in the assessment of AD, extent and subjective symptoms account for around 20% each of the total score, intensity items representing 60%. The so-designed composite index SCORAD needs to be further tested in clinical trials.

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INDOOR AIR POLLUTION AND ALLERGEN EXPOSURE IN HOMES OF ATOPIC ECZEMA PATIENTS. Barbara Kunz[1], Yves de Prost[2], Johannes Ring[1], [1]Department of Dermatology, University Hospital Eppendorf, University of Hamburg, Germany and [2] Hopital Necker Enfants Malades, University of Paris, France

In face of increasing evidence for a role of environmental factors in the pathophysiology of atopic eczema (AE), we have performed a case-control study in 57 AE patients (age 4m - 9y) and 18 controls (age 8m - 10y). Specific sensitization to 6 common allergens was determined by prick test and RAST in all patients. The childrens' domestic environment was monitored by means of passive samplers for airborn SO_2, NOx and formaldehyde (FA) and evaluation of house dust mite allergen levels (Der P1 and Der F1, ELISA technique). The concentrations of SO_2, NOx and formaldehyde in Parisian homes were 28,9 (SO2);20,8 (NOx);7,0 (FA) [$\mu g/m^3$],medians). There were no differences in exposure levels between atopic and control childrens' bedrooms. Der P1 is found in 80% of atopics'(median 160 ng/g dust) and 60% of controls'(199 ng/g dust) homes, without significant differences between the two groups. Atopic children having specific serum IgE to D. pter. were exposed to significantly higher levels of Der P1 (2509 ng/g dust) than were those without sIgE (450ng/g dust). The present study does not show differences in indoor environment between atopic patients and controls. Specific house dust mite sensitization in AE patients is related to house dust mite allergen exposure levels.

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CONCORDANCE OF ATOPY PATCH TEST, PRICK TEST AND SPECIFIC IgE IN PATIENTS WITH ATOPIC ECZEMA. Ulf Darsow, Dieter Vieluf and Johannes Ring, Department of Dermatology/Allergology, University of Hamburg, Hamburg, FRG

In a subgroup of patients with atopic eczema (AE) eczematous skin lesions can be induced by epicutaneous testing with aeroallergens (Atopy Patch Test, APT). Previous studies revealed intraindividual differences in reactivity to APT, prick test and amount of specific serum-IgE to the allergens tested.

We performed a standardized APT in 53 patients with moderate to severe AE. Patients were tested epicutaneously on non-abraded, uninvolved back skin with allergens of house dust mite (D.pter.), cat dander and grass pollen in a concentration of 10.000 PNU/g (vehicle: petrolatum). Reactions were evaluated after 48 hrs. In order to correlate the classic diagnostic parameters of IgE-related allergy with the APT, prick tests and determination of total and specific IgE to the 3 allergens have been performed. Clear-cut positive APT-results were compared to the individual prick test and RAST-results (CAP-class >1).

23 of 53 patients with AE showed at least one clear-cut positive APT reaction, 14 of them had a total IgE > 1,000 U/l. 87% of these 23 patients were found to be reactive to D.pter., 61% to cat dander and 35% to grass pollen. In 75% of patients presenting positive APT to D.pter., a corresponding positive prick test (cat: 93%, grass pollen: 63%) and in 80% of them a corresponding RAST (cat: 71%, grass pollen: 75%) could be observed. 15 of 34 (16 of 30) patients with a D.pter.-positive prick test (RAST) showed a corresponding positive APT-result, but also 5 of 19 (4 of 23) prick-negative (RAST-negative) patients developed clear-cut eczematous APT reactions with this allergen.

The results show that positive intracutaneous tests and raised allergen-specific IgE are not mandatory for a positive APT. It can be concluded that the APT may give further diagnostic information in addition to the established test procedures to elucidate the relevance of aeroallergens eliciting eczematous skin lesions in a subgroup of patients with AE.

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METHODOLOGICAL ASPECTS OF DIAGNOSTIC MODELS OF ATOPIC DERMATITIS. Thomas L. Diepgen, Willi Sauerbrei*, Manige Fartasch, Dept. of Dermatology Univ. of Erlangen, *Inst. of Medical Biometry Univ. of Freiburg

Since there is no objective laboratory marker for atopic dermatitis (AD), the diagnose should be based on an array of anamnestic and clinical features. The goal of our study was to evaluate and validate a diagnostic model for epidemiological research. *Evaluation study:* In a case-control-study the diagnostic value of atopic features and laboratory findings were systematically studied in established cases of AD (n=345) and in non-eczematous controls collected from the general population (GP: n=618). The aim was to model an easy to use score, which might help to establish a firm diagnosis of AD in epidemiological studies. Two different logistic regression models were analysed to evaluate the diagnostic value of atopic features. In the first model a diagnostic score "A" (without constraints) was constructed after selection (backward elemination technique), consisting of 12 variables out of 21 atopic features. With this score both groups (AD versus GP) are seperated fairly well with minimal overlapping. In a second step we defined a score "B" which was based only on 10 out of 15 "hard criteria" with good interobserver agreement (model under constraints). The backward elimination technique showed that in the multivariate framework 5 variables did not contribute to separation of the 2 groups. With the ten remaining variables we defined a very easy to use score whose correlation with the first score with variables suspected to measurement error was very high (r=0,93). *Validation study:* To validate the proposed diagnostic models with new data a second multicenter study was performed (n=329). These results will be compared to the proportion of incorrect classification from the original data set where an attempt was done to estimate the true error of incorrectly predicting the diagnosis on a bootstrap approach. Additionally a validation of the original data with computer intensive methods (cross-validation, Jackknife, Bootstrap) was performed. This study shows that estimates from original data are biased because of complex modelling, error rates are to optimistic, and Jackknife approach does not offer a better estimation of the true error rates. Whereas in the original data the fit of the model without constraints is much better than the fit of the models under constraints, this is no longer true for the validation data: the simplified score "B" gives high sensitivity (90%) and specificity (88%). Therefore validation studies for multivatiate modelling in epidemiological research are necessary.

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ANXIETY, DEPRESSION, PSYCHOSOMATIC SYMPTOMS AND AUTONOMIC NERVOUS FUNCTION IN PATIENTS WITH CHRONIC URTICARIA. Makoto Hashiro and Mutsuko Okumura, Department of Dermatology, Minoh City Hospital, Osaka Japan.

Most reports on the psychological analysis of chronic urticaria have concentrated on psychodynamic theories of causation of the disease. For the present study, we used three kinds of psychological tests as well as electrocardiography to estimate anxiety, depressiveness, psychosomatic symptoms and autonomic nervous functions in 30 outpatients with chronic urticaria and 39 normal controls. For evaluation we used the manifest anxiety scale (MAS), self-rating depression scale (SDS), Cornell medical index (CMI) and convergence of variance of R-R interval (CV_{R-R}). Stages 1 and 2 on the MAS, scores not less than 40 on the SDS and areas 3 and 4 on the CMI were observed in 40.0%, 43.3% and 40.0% of the chronic urticaria patients. These findings respectively compared with 15.4%, 12.8% and 10.2% of the controls. Psychologically positive responses to any of the tests were seen in 70.0% of the chronic urticaria patients, but only 25.6% of the controls. These differences all showed statistical significance ($p<0.01$). The ln (CV_{R-R}) [Y] and age [X] suggested a linear regression, but although the regression slope was steeper for the urticaria group ($Y=2.924-0.027X$) than for the controls ($Y=2.702-0.023X$), the difference was not statistically significant.

These data indicate that patients with chronic urticaria are more anxious, depressive and psychosomatic symptom-prone than normal control. In conclusion, we suggest that chronic urticaria patients should be diagnosed and treated both dermatologically and psychologically.

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SQUAMOUS CELL CARCINOMA ANTIGEN ELEVATED IN PUSTULAR PSORIASIS. Mayumi Ujihara, Sumiko Hamanaka, Department of Dermatology, Yamaguchi Rosai Hospital, Onoda, Yamaguchi 756, Japan.

The squamous cell carcinoma (SCC) antigen has been developed as a tumor marker for SCC of the uterine cervix, but it is also elevated in the serum of patients with non-carcinomatous dermatoses. Whether the two SCC antigens are the same or not requires examination. We investigated the serum SCC antigen levels of 34 patients with psoriasis. Using isoelectric focusing, we analyzed the serum SCC antigen detected in a patient with pustular psoriasis (he showed the highest level in our study), and examined the tissue localization of SCC antigen. We also followed the change in its serum level after the effective medication of cyclosporine. The mean serum SCC antigen level of patients with psoriasis was 6.23 ± 2.72 ng/ml, which is much higher than the cut-off value of 1.5 ng/ml. The isoelectric focusing pattern demonstrated both the neutral and the acidic fraction, and the positive staining of macrophages in the dermis was observed with the monoclonal antibody specific to the acidic fraction. The serum level of SCC antigen reflected the disease activity well. Generally, the neutral fraction is present in both the malignant and non-malignant squamous cells, whereas the acidic fraction is increased mainly in malignant cells. It is interesting to have detected the acidic fraction in the serum of the patient with pustular psoriasis, and further investigation is desired to clarify the mechanism of the expression of SCC antigen.

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AGN 190168, AN INVESTIGATIONAL RETINOID GEL FOR THE TREATMENT OF PLAQUE PSORIASIS. J. Sefton, D. Lew-Kaya, J. Lue, R. Chandraratna, and the Topical Retinoid and Psoriasis Research Group, Departments of Clinical and Discovery Research, Allergan Herbert, Irvine, CA, USA

AGN 190168, a member of a novel class of acetylenic retinoids, is a potent inhibitor of ornithine decarboxylase activity and is being investigated for the treatment of plaque psoriasis. In a molecular marker study, AGN 190168 0.05% gel, applied twice daily for 2 weeks, produced a more normal expression of skin differentiation and inflammatory markers in psoriatic patients. In an initial, blinded, clinical study, twice-daily application of 0.05% gel for 6 weeks resulted in a significantly higher rate of treatment success (good, excellent, or clearing response) compared with vehicle: 45% vs 13%, $p< 0.05$. A subsequent dose-ranging study compared two concentrations of AGN 190168 (0.05% and 0.1%) and two dosing regimens (q.d. and b.i.d.). After 8 weeks, treatment success rates were 48% for 0.05% q.d., 63% for 0.05% b.i.d., 57% for 0.1% q.d., and 60% for 0.1% b.i.d. In many patients, therapeutic effect was sustained throughout an 8-week post-treatment phase, and some patients continued to improve post-treatment. A percutaneous absorption study, in which radiolabeled AGN 190168 0.1% gel was applied under occlusion, demonstrated a total systemic absorption of less than 6%. Side effects in clinical trials have been those commonly associated with topical retinoids (erythema, pruritus, burning), have generally been mild to moderate in severity, and have affected 13% to 30% of patients in a dose-related fashion. Urinalysis, hematology, and blood chemistry results have shown no significant drug effects. These promising preliminary results indicate the need for further investigation of AGN 190168 in the treatment of psoriasis.

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HIGHLY PURIFIED OMEGA-3-POLYUNSATURATED FATTY ACIDS FOR TOPICAL TREATMENT IN PSORIASIS - RESULTS OF A DOUBLE-BLIND, PLACEBO-CONTROLLED MULTICENTRE STUDY. H.-H. Henneicke-von Zepelin, U. Mrowietz, E. Christophers, D. Welzel, and L. Färber, Dept. of Dermatology, Univ. of Kiel and Clinical Research Department, Sandoz AG Nürnberg, FRG.

Fish oil contains large amounts of omega-3-polyunsaturated fatty acids (Omega-3-PUFA). Omega-3-PUFA were reported to have beneficial effects on psoriatic lesions when applied topically (Dewsbury et al., Br J Dermatol, 1989, 120: 581-584) as determined by an open, placebo-controlled study. The present multicentre, doubleblind, placebo-controlled study was conducted in order to investigate efficacy, safety and tolerability of topically applied, highly purified omega-3-PUFA in two different concentrations (1%, 10%). A total of 52 patients suffering from chronic plaque form psoriasis were enrolled into the study. Patients with severe organe dysfunction, hypertension, clinically relevant allergies and metabolic disorders were excluded. Two similar stable psoriatic lesions served as indicator lesions. After one week run-in phase, treatment with omega-3-PUFA (1% or 10%) or placebo was randomly assigned to the selected lesions. Efficacy was assessed according to changes in local psoriasis severity index, area involved, erythema, desquamation, induration and pruritus. Patients' compliance was closely monitored.

After 8 weeks of treatment, all indicator lesions improved significantly compared to baseline. However, no statistically or clinically relevant differences between the different treatment groups were found. Safety and tolerability of the preparations tested were good (only one patient showed perilesional eczema, another clinically relevant adverse event did not occur).

In conclusion, omega-3-PUFA in topical use failed to be efficacious in a randomized, placebo controlled, double blind setting. Results of unblinded trials should be (re-)considered with caution.

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CRABP2 mRNA LEVELS AND KOEBNER PHENOMENOM IN NONLESIONAL PSORIATIC SKIN. **Liliane Didierjean, Robert Feldmann, and Jean-Hilaire Saurat, Department of Dermatology, University Hospital, Geneva, Switzerland.**

We have previously found a sharp increase of CRABP protein and overexpression of CRABP2 mRNA in lesional skin of psoriatic patients (LPS) as compared to nonlesional (NLPS) and normal skin (NHS). However CRABP2 mRNA was already overexpressed in some specimens of NLPS which were histopathologically normal. Koebner phenomenon (KP) is an "all or no" event; there is so far no predictive biological marker for a Koebner-prone NLPS. We wondered if overexpression of CRABP2 might be such a marker.

Fifteen patients were prospectively studied. NLPS were keratomized on buttocks at a distance of >10 cm from any plaque. Patients were reexamined for Koebner reaction weekly for one month and twice a month for the two following months. The site was quoted 0-3 for erythema induration and scales. CRABP2 mRNA expression was studied by Northern blots with keratin 14 probe as control and quantified by slot blots.

Out of 15, 6 patients (40%) showed a KP on the keratome site, in all 6 CRABP2 transcripts were identified contrasting to no detection in NHS. In 9 (60%), no KP occured; CRABP2 mRNA were also detectable in all specimens. Relative quantification by slot blots did not show differences in the amount of CRABP2 mRNA in KP (+) as compared to KP (-) group [(mean arbitrary units) KP (+) : 4413±1541, KP (-) : 3793±1495]. There was no correlation between the intensity of KP and the amount of CRABP2 mRNA.

This study confirms that CRABP2 mRNA is higher in NLPS as compared to NHS; it is not known whether this corresponds to increased transcription or stabilisation of mRNA. As CRABP2 protein is also increased in NLPS an alteration of retinoic acid metabolism is a feature of NLPS. However this does not appear to correspond to propensity toward developing KP upon wounding.

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HETEROGENEOUS DISTRIBUTION OF CYTOCHROME P-450 I A1 IN THE PSORIATIC EPIDERMIS. Mariko Iizuka, Yo Kawakubo, Itsuro Matsuo, Muneo Ohkido, Department of Dermatology, Tokai University School of Medicine, Isehara, Japan

The cytochrome P-450 monoxygenase system plays an important role in the oxidative metabolism of many drugs, xenobiotics and some endogeneous substances. It is also considered that CyP-450 I A1 is one of markers for the epidermal differentiation. On the other hand, in the hyperproliferating state, the amount of CyP-450 decreased in the liver. Therefore, cellular proliferation and differentiation may affect CyP-450. Moreover, in porcine ciliary epithelium, CyP-450 I A1 was recently reported to catalyze arachidonic acid metabolism to 12-R-hydroxy eicosatetraenoic acid (12-R-HETE), which is abundant in the psoriatic epidermis. In cultured human epidermal keratinocytes, CyP-450 I A1, I A2 II B1 and III A4 are reported to exist. We have immunohistochemically investigated the distribution of CyP-450 I A1 and I A2 in the normal and the psoriatic skins as well as epidermal neoplasms. CyP-450 I A1 and I A2 were identified in the normal epidermis. The negative staining in the lower epidermis in psoriatic subjects and the weak staining in the epidermal neoplasms suggest that the disturbance in differentiation and/or proliferation may exsist. The heterogeneous distribution of CyP-450 I A1 in the psoriatic epidermis overlaps apparently with that of 12-R-HETE which is reported previously, implying that the CyP-450 I A1 in the epidermis may catalyze metabolism of arachidonic acid to 12-R-HETE.

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EOSINOPHIL CATIONIC PROTEIN IN PSORIASIS. Masahiko Toyoda, Tomohiro Maruyama, Taisuke Seki and Masaaki Morohashi, Department of Dermatology, Faculty of Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan

Blood eosinophilia is rarely observed in psoriasis and little attention has been paid to the infiltration of eosinophils into the psoriatic skin lesions, hence the role of eosinophils in psoriasis has not been elucidated. The purpose of this study is to clarify the role of the eosinophil and eosinophil cationic protein (ECP), one of the eosinophil granule proteins, in psoriasis. ECP levels in serum samples of sixty-four patients and ten healthy individuals were measured by means of a double antibody radioimmunoassay. Skin biopsy specimens taken from twenty-three patients were stained by two kinds of monoclonal antibodies to ECP (EG_1 and EG_2) using the Avidin-Biotin-immunoperoxdase staining method.

In the psoriasis group, the levels of serum ECP were significantly higher than those of the control group. The levels of serum ECP in patients with psoriasis were statistically elevated in the active state compared with those in the inactive state. In the immunohistological study, 4 out of 6 cases in the active state and 1 out of 17 cases in the inactive state showed strong expression of both EG_1 and EG_2. Although EG_2 immunoreactivity was found mainly in the cytoplasm of eosinophils in the upper dermis, EG_1 immunoreactivity was found not only in the upper dermis but also in the epidermis in various patterns

These findings suggest that the eosinophil and ECP may be relevant to the aggravation of psoriasis.

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SELF-RATING OF PSORIASIS DISEASE ACTIVITY. SR Feldman, AB Fleischer, Jr, J Vanarthos, and SR Rapp, Departments of Dermatology, Pathology, and Psychiatry, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC, USA

Assessing the severity of psoriasis disease activity is difficult. The Psoriasis Area and Severity Index (PASI) can be utilized by health care providers, but no effective method exists for patients to evaluate their own condition. A new instrument was developed for patients to self-assess their psoriasis. The instrument consisted of a silhouette of a body for patients to shade in affected areas and of three modified visual analog scales for recording the redness, thickness, and scaliness of an average lesion. The resulting "Patient PASI" score was compared to provider PASI measurements in 29 patients with mild to severe psoriasis. Patients and providers also recorded a subjective overall severity score. The Patient PASI score was highly predictive of the provider PASI score ($r=0.86$, $p<0.0001$) and the provider overall score ($r=0.83$, $p<0.0001$). The patients' overall assessment was also predictive of the provider PASI score ($r=0.57$, $p=0.0013$) and the provider overall score ($r=0.64$, $p=0.0002$), but these relationships exhibit wide confidence intervals. We conclude that patients' overall scores do not allow for accurate prediction of clinical disease severity (as measured by provider PASI scores), but a structured instrument allows patients to produce a surprisingly accurate measure of their disease activity.

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SCH 40120, A Novel Leukotriene and Cytokine Inhibitor, in the Treatment of Psoriasis Vulgaris

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SCH 40120, a novel naphthyridinone compound, inhibits in vitro production of leukotriene B4 and C4, IL-1a and TNF-a, and is efficacious in animal models of acute inflammation. The topical activity of this drug is under study in hyperproliferative and inflammatory dermatoses. Cutaneous tolerance of the drug following t.i.d. application times two weeks in normal volunteers has been demonstrated. In a bilateral-paired comparison trial SCH 40120 1% cream and vehicle were applied t.i.d. for six weeks in 20 patients with psoriasis vulgaris. At the end of treatment, SCH 40120-treated plaques exhibited 73% improvement (mean) versus 34% and 8% improvement in the vehicle and non-treated plaques, respectively ($p<0.01$). The drug was well tolerated locally and no clinically significant abnormalities in clinical laboratory evaluations were detected. Further evaluations are underway to investigate the role of SCH 40120 in the treatment of psoriasis and other inflammatory dermatoses.

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BONE MINERAL DENSITY IS UNAFFECTED BY PALMOPLANTAR PUSTULOSIS. Gregor B.E. Jemec[1],Gina Kollerup[2],Peter Nymann[1],Edith Grossman[3], [1]Dept. of Dermatology, Bispebjerg Hospital,[2]Dept. of Medicine,and [3]Dept. of Radiology, Sundby Hospital, Copenhagen, Denmark

Lumbar sclerosis and thoracic ossification have been described in patients suffering from palmoplantar pustulosis (PPP). The changes are found in X-ray examination. It is unknown if the X-ray findings only represent the end stage of skeletal involvement, or if more subtle changes occur in the bone mineral density (BMD) of PPP patients. The BMD of 12 female patients (median age 65.5, 95%CI:47-75 yrs) was measured using an X-ray bone densitometer (Norland XR26, Wisconsin). The median history of PPP was 12(2-15) years. Disease severity was scored by counting pustules in the right palm and left sole, and by assessing the percentage of the palm and sole showing erythema and scaling (3 scores each, total range 3-9). No skeletal abnormalities were found in an X-ray examination of the thoracic skeleton of these patients. BMD was measured over the lumbar spine (L2-L4), and the results were compared with those of the background population (relative BMD,%). The score was 3.5(3-6), the BMD was 0.9235(0.6430-1.080), and the relative BMD was 105.45%(74.8-122.3). No statistically significant differences were identified, suggesting that BMD is unaffected in this group of PPP patients and that skeletal involvement may be due to a specific mechanism not universally present in PPP patients.

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THE NEW MODEL OF TRANSEPIDERMAL ELIMINATION IN CHROMOMYCOSIS.

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In chromomycosis, sclerotic cells are often found on microscopic examination of the skin scales, and it is known that there is a mechanism for the transepidermal elimination of these cells. Therefore, we used a new experimental model to investigate this phenomenon. The agar implantation method previously used in the peritoneal cavity (Miyaji & Nishimura : Mycopathologia 1977) was applied to the skin. Fonsecaea pedrosoi was used as the test strain, and was prepared in agar blocks. This agar blocks were then implanted into the dermis on the backs of C57BL/6 mice. Thereafter, skin specimens including the implanted agar blocks were collected sequentially and were examined histologically. Two weeks after implantation infiltraion of inflammatory cells was observed around the agar block, and granuloma formation was noted from the 4th week. After 6 weeks epidermis was seen extending to the agar blocks surrounded by infiltrating cells, and transepidermal elimination of the organism was observed. However, when the experiment was performed using beige mice of the same strain, only cellular infiltration was found and no transepidermal elimination was observed. These findings suggested that the excretion of sclerotic cells in chromomycosis occurs stimulating the epidermis via cellular infiltration, especially NK cells. The intradermal agar implantation method is useful not only for demonstrating the histological findings at an early stage (which are rarely observed), but also for clarifying the mechanism of transepidermal elimination.

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EVALUATION OF A NEW SYNTHETIC ANTIMICROBACTERIAL AGENT, OPC-7251 CREAM, IN THE TREATMENT OF ACNE VULGARIS WITH MODERATE OR SEVERE INFLAMMATORY ACNE LESIONS : A MULTICENTER, DOUBLE-BLIND, GRUOP COMPARATIVE STUDY WITH A CREAM BASE.

Ichiro Kurokawa (OPC-7251 Acne Study Group), Department of Dermatology, Hyogo Prefectural Tsukaguchi Hospital, Hyogo, Japan

Clinical efficacy of a topical antimicrobacterial agent, a 1% cream of OPC-7251, was determined in patients with acne vulgaris in comparison with its base at 14 institutions in a double-blind, group comparative manner.

A total of 112 patients entered the study, and 48 and 55 evaluable patients were included in statistical evaluation of OPC-7251 and its cream base, respectively. The test materials were topically applied twice daily to areas of acne after washing the face for four weeks. For evaluating the clinical usefulness, the number of inflammatory lesions were counted, and the severity of inflammation was obtained on a 5-grade scoring system, before and at 1, 2, 3 and 4 weeks of treatment. Clinical usefulnesswas assessed by global judgement of efficacy and safety in 104 patients, 48 and 56 in the active treatment and control groups, respectively, and the treatment was assessed as useful (useful and very useful) in 39 (81.3%) patientsin the active treatment and in 17 (30.9%) of the controls, indicating the superiority ($p=0.0000$) of OPC-7251. Adverse reactions reported were redness in 1 (2.0%) in OPC-7251 group, demonstrating that OPC-7251 was a very safe drug. Thetherapeutic value of a 1% cream of OPC-7251 in acne vulgaris was thus demonstrated in the present double blind study using patients with multiple inflamed acnelesions.

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STUDY OF RESIDUAL EFFICACY OF ANTISEPTIC HANDRUB LOTION WITH AN AUTO-STERILIZER. Shoko Namura, Setsuko Nishijima and Yasuo Asada, Department of Dermatology, Kansai Medical University, Osaka, Japan

We used the full-hand touch plates method to evaluate the residual effect of quick handwashing by means of a 3-second application of antiseptic handrub lotion with an auto-hand-sterilizer. 10 subjects were instructed not to use any antimicrobial agents for 2 weeks and not to wash their hands for 5 hours before the test. They stamped their palmers on the large plates of agar before washing their hands (1st), and immediately after washing (2nd), and then they instructed to touch things under normal habits and conditions. The following sampling times were 10 minutes (3rd), 30 minutes (4th) and 2 hours (5th) after handwashing. The test lotion was WELPAS® , applied after 30 seconds of handwashing with soap. WELPAS®, available as a quick-dry handrub lotion, showed about 80% reduction against hand surface bacteria just after use, but seemed not to have sufficient residual effect under various conditions of contamination, showing no better effect than washing with soap alone after 10 minutes, 30 minutes, or 2 hours.

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INCREASED EXPRESSION OF EXTRACELLULAR PROTEOLYTIC ACTIVITY IN *CANDIDA ALBICANS* ISOLATES FROM THE ORAL CAVITIES OF HIV-INFECTED PATIENTS. Hans Christian Korting, Christine Goebel, and Markus Ollert, Dermatologische Klinik und Poliklinik, Ludwig-Maximilians-Universität München and Department of Biochemistry and Molecular Biology, University of Hamburg, Germany.

C. albicans secretory protease (CAP), an extracellular acidic protease, represents an important virulence factor in the development of mucocutaneous candidosis. Current experimental knowledge leads to suggest, that CAP plays a major role in tissue invasion by *C. albicans* through proteolytic cleavage of host cell proteins in the microenvironment zone of close parasite-host cell contact. Up to now, the increased prevalence of oropharyngeal candidosis and the severeness of the apparent clinical manifestations in HIV patients were attributed exclusively to the virus-induced T-cell defect of the host. The present study was aimed to answer the question whether Candida derived virulence factors have any potential influence on the clinical manifestations of oropharyngeal candidosis seen in HIV patients. Thus, we measured CAP activity of clinical *C. albicans* isolates from the oropharynx of either HIV-positive individuals (n=119) or a control group (n=122). For determination of enzyme activity from culture supernatants, *C. albicans* isolates were grown in Remold medium for 7 days. CAP activity was quantitated with the Folin method in a spectrophotometer after proteolysis of hemoglobine substrate at pH 6.5 and subsequent TCA precipitation of soluble proteins. An increase in the $OD_{700\ nm}$ over a period of 60 min was translated into CAP activity units. Mean CAP activity of *C. albicans* isolates from the HIV group (4256 U/L) was significantly higher compared to the control group (2324 U/L) as tested by the Mann-Whitney test ($p \leq 0.0001$). Of the *C. albicans* isolates derived from HIV patients, 47% belonged to a group of high CAP activity (≥4000 U/L), 32% expressed medium CAP activity (≥2000<4000 U/L), and 21% low CAP activity (<2000 U/L). Isolates of the control individuals, however, showed an almost inverse distribution among the 3 groups: 8%, 41%, 51%, respectively. Our data allow the conclusion that *C. albicans* isolates from the oropharynx of HIV patients express significantly higher CAP activity in comparison to a control group. To what extent this protease activity pattern correlates with the increased expression of the *C. albicans* serotype B in HIV patients will need further investigation.

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A CLINICAL AND LABORATORY STUDY OF CICLOPIROX OLAMINE (8% BATRAFEN) IN THE TREATMENT OF ONYCHOMYCOSIS

Yu Baotian M.D., Zhou Guangji M.D., Wang Baoxi M.D., Ben Yaxin M.D., Yan Hong, Shao Yanling and Wang Baoming, Department of Dermatology, Peking Union Medical College Hospital, Beijing, People's Republic of China

Ciclopirox olamine, a new synthetic substituted pyridone derivative, is an antimycotic agent with activity against a broad spectrum of pathogenic and non-pathogenic fungi. The clinical efficacy and safety of 8% ciclopirox olamine nail liquor were evaluated in 100 cases with finger (or great toe) onychomycosis. After 16 weeks and 24 weeks of treatment for finger and great toe onychomycosis, respectively, the overall therapeutic results were excellent in 36 cases, good in 17, fair in 24, and poor in 25. The period of treatment was extended in 31 cases; among them, 10 cases showed further improvement. As for *in vitro* inhibitory activity, the MIC of ciclopirox olamine against *T. rubrum* and *C. albicans* was 1 to 4 mg/L and 1 to 16 mg/L, respectively. This study indicates that 8% ciclopirox olamine nail liquor, with its satisfactory efficacy and lack of side effects, is a good remedy for onychomycosis.

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EVALUATION OF BACTERIAL INDEX (BI) IN LEPROSY PATIENT USING DNA HYBRIDIZATION. Yasuyuki Sugita, Shutaku Kim, Norihisa Ishii and Hiroshi Nakajima, National Sanatorim Tama-Zenshoen, Tokyo and Department of Dermatology, Yokohama City University School of Medicine, Yokohama, Japan

Skin smear examination tests in leprosy patients have been very important for monitoring the disease activity and effect of drug treatment. Bacterial index (BI) is an indicator of bacterial density in patients' tissue. It ranges from zero to 6+ and is based on the number of bacilli evident microscopically after acid-fast staining. However, observation of acid-fast-stained M.leprae sometimes requires experience, and the evaluation may vary among different examiners. In this study, we attempted quantitative evaluation of the bacterial load in leprosy patients using DNA hybridization.

We generated a M.leprae-specific DNA probe by polymerase chain reaction using specific PCR primers from the gene encoding heat shock protein 70. Equivalent amounts of the DNA extracted from the whole skin of leprosy patients were subjected to DNA hybridization using the M.leprae-specific DNA probe.

Our results demonstrated a correlation between the BI count and density of hybridization, indicating the reliability of quantitative hybridization analysis for accurate evaluation of bacterial load in tissue.

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CONDYLOMATA ACUMINATA RESPONSES TO INTRALESIONAL MPI 5003 AND THE SHOPE RABBIT PAPILLOMA MODEL TO EVALUATE DRUG MECHANISMS. Elaine K. Orenberg and John W. Kreider, Matrix Pharmaceutical, Inc., Menlo Park, CA and Department of Pathology, The Milton S. Hershey Medical Center, Hershey, PA, U.S.A.

A new technology for local delivery of fluorouracil is under development to provide enhanced therapeutic drug levels at the treatment site. We evaluated the safety and efficacy of intralesional chemotherapy with MPI 5003 in a multicenter phase II, double blind, placebo-controlled study for the treatment of external condylomata acuminata in 200 patients. MPI 5003 is a viscous injectable gel, comprised of a collagen-based carrier matrix, epinephrine and fluorouracil. The condyloma response rate, duration of response and recurrence rate were described and evaluated for MPI 5003, in comparison to MPI 5003 without epinephrine and placebo. We further evaluated the mechanisms by which MPI 5003 eliminates condylomata using the Shope papilloma model in which rabbit papillomas have a viral genome analogous to HPV.

Condyloma treatments were given once weekly to each lesion for 6 weeks; maximum 150 mg fluorouracil per week. Efficacy endpoint was complete response (CR) of all treated warts/patient. For animal studies, we assessed papilloma growth inhibition as a function of initial virus inoculum and MPI 5003 dose of lesions of various sizes using a treatment regimen similar to human studies. We also determined mitotic indices and viral DNA replication by Southern transfer analyses.

A total of 187 patients with 858 lesions were evaluable. All warts per patient were treated. Most patients (74%) had total wart areas of <100 mm^2 in which MPI 5003 produced an 80% CR. Patients with total lesion areas of 1 to 532 mm^2 had a CR rate of 65%. Patients with CRs were followed for 3 months, 61% remained disease free. The Shope model was useful to study intralesional MPI 5003 since lesions were responsive over a range of drug doses and papilloma virus inoculum. MPI 5003 produced direct papilloma growth inhibition and cures. No viral DNA was detected in cured lesions.

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IMPAIRED ANTIGEN PRESENTATION IN TOXIC EPIDERMAL NECROLYSIS. Dominique Charue, Jean-Claude Roujeau, Michèle Heslan, Janine Wechsler, Jean Revuz, and Martine Bagot, Departments of Dermatology, and Research in Immunodermatology, Henri Mondor Hospital, Université Paris XII, Créteil, France.

The pathophysiology of toxic epidermal necrolysis (TEN) remains largely unknown. TEN is considered to be a hypersensitivity reaction to drugs, but direct evidence of an immunologic mechanism is still lacking. We investigated several T-cell functions in a series of 11 patients with TEN. Peripheral blood mononuclear cells (PBMC) obtained in the acute phase were tested together with PBMC obtained after the patient's recovery, and compared with those of age- and sex-matched healthy controls. PHA-induced proliferations and lymphocyte responses in allogeneic mixed lymphocyte reactions were not impaired in acute phase, when compared to the same patients after recovery, and to controls. In contrast, Natural Killer cytotoxicity and allogeneic cytotoxic responses were significantly decreased in early TEN. The most striking feature was the significantly impaired ability of acute phase lymphoid cells to activate allogeneic T cells. Patient PBMC in acute phase did not inhibit the proliferation induced by patient PBMC after recovery, suggesting that their defect was not related to the presence of radioresistant suppressor cells. The phenotypic expression of HLA-DR, -DQ, and -DP antigens on circulating PBL was then assessed by immunoalkaline phosphatase staining and flow cytometry. Results showed decreased percentages of HLA-DR positive mononuclear cells and a decreased density of HLA-DR antigens, mainly on monocytes, in acute phase.These results demonstrate that peripheral blood lymphocytes of patients with TEN have an impaired ability to activate allogeneic T cells. This defective antigen presentation is not due to the presence of suppressor lymphocytes, but is probably related to a decreased expression of HLA-DR antigens on circulating mononuclear cells in acute phase, either induced by cytokines, or secondary to the migration of activated PBMC to lesional skin.

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CROSS HYPERSENSITIVITY TO IONIZED MERCURY AND NOT TO NON-IONIZED MERCURY WITH GOLD DERMATITIS PATIENTS CAUSED BY EAR PIERCING. Tokio Nakada*, Masafumi Iijima*, Ryuichi Fujisawa* and Hideo Nakayama**, *Department of Dermatology, Showa University School of Medicine, Tokyo, Japan **Department of Dermatology, Saiseikai Central Hospital, Tokyo, Japan

25 female patients with gold dermatitis (GD) due to ear piercing with no apparent history of mercury hypersensitivity along with 5 systemic contact dermatitis patients due to mercury (baboon syndrome) were closed patch tested with 0.2% $HAuCl_4$, 0.05%$HgCl_2$, the both in aqueous solutions, and 0.5% Hg in petrolatum. The former mercury was ionized with a pair of outermost free electrons lost, and the latter mercury was not ionized with the free electrons preserved. The patch test was performed for two days, and the reading was done on the days 2,3 and 7 usually.

	$HAuCl_4$	ionized Hg	non-ionized Hg
GD	25/25(100%)	17/25(68.0%)	2/14(14.3%)
SCD	0/5 (0%)	5/5 (100%)	5/5 (100%)

There was a statistical significance between the positive rates of ionized mercury (68%) and non-ionized mercury (14.3%) with gold dermatitis. On the other hand, all the SCD patients positively reacted to both ionized and non-ionized mercury, but none to Au^{3+} ions. These results suggest that cross hypersensitivity to Hg^{2+} and not to non-ionized Hg was present with the contact dermatitis patients already sensitized by Au^{3+} ions, because of the similarities of the electron structures of Hg^{2+} and Au^{3+} ions. Those who were originally sensitized by Hg seemed to have had another form of determinant derived from Hg or Hg^{2+} which did not cross react to Au^{3+} ions.

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A NOVEL PREDICTIVE TESTING FOR CONTACT ALLERGY

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Although the maximization was utilized as predictive testing to forecast allergic potential of chemicals this method is timeconsuming and difficult to evaluate contact sensitivity quantitatively.In the present paper, we established a simple and quantitative predictive test to detect potent allergens. Monolayered Pam212(Keratinocyte cell line)cells were incubated with a substance which should be evaluated allergenic potential , washed and fixed. T cells(TC) and macrophages from nonsensitized Balb/c mice were cocultured with these monolayered substance conjugated Pam cells for 5 days, harvested and restimulated with substance cojugated spleen cells(MMC treated) in 96 wells culture plate . 3 strong sensitizers(Oxazolone ,TNCB ,DNCB),3 potent sensitizers (p-phenylendiamine, Nickel chloride, ,Potassium dichromate),2 corticosteroids (betamethasone,budesonide) and methyl salicylate(MS) were evaluated. Stimulation Index(SI) of strong sensitizers was around 400%. SI of potent sensitizer(P-PD) was 236%. SI of budesonide, betamethasone,MS were 162, 98,121%. These results indicate this novel method could be a useful predictive testing for contact allergens.

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ALLERGY DIAGNOSIS IN PATIENTS WITH ADVERSE REACTIONS TO ANALGESICS: FOLLOW-UP INVESTIGATION. A. Laking, D. Vieluf, K.H. Schulz, J. Ring, Department of Dermatology and Allergology, University of Hamburg, Germany

Mild analgesics and non-steroidal antiinflammatory drugs (NSAIDs) containing e.g. salicylates, pyrazolones, p-aminophenoles, diclofenac as main active compounds are well-known elicitors of anaphylactoid reactions (AR) and other adverse reactions including a variety of exanthemas (EX). We assessed the diagnostic value of case histories (n=352), skin tests [i.c./patch (n=338/228)] and oral provocation tests [OPT (n=106)] in patients with AR (n=315) or EX (n=37) to analgesics and/or NSAIDs (sex: f=229, m=123; age: 7-85 y.; 83 atopics). In addition we analyzed the follow-up using a katamnestic questionnaire mailed to all 352 patients (40% response rate, n=142), with regard to newly occuring adverse reactions to analgesics and/or NSAIDs (time interval: 0,5 - 3,5 y.). 26/338 patients (8%) showed clear-cut positive reactions (>= ++) in the i.c. test with a standard battery of analgesics/NSAIDs and 22/228 (10%) (>= +) in the patch test. Patients with a severe AR to analgesics and/or NSAIDs in the case history yielded statistically significant more >= ++ reactions in the i.c. to propyphenazone. 17/72 (23%) selected patients reacted in the OPT to a single component of the culprit preparation, and 8/75 (11%) showed an AR in the OPT to an unsuspicious analgesic/NSAID. 91/142 patients reused analgesic/NSAID preparations after the allergological examination. 10/91 (11%) showed an AR to the administered drug: 1 patient reacted to the accidentally reused known elicitor of the previous AR, 2 reacted to the drug tolerated in the OPT and 7 reacted to analgesic preparations not tested by the OPT. From these data we conclude that the oral provocation test takes its place next to a comprehensive history in diagnosing the elicitor of an anaphylactoid reaction. Furthermore, OPTs are indicated to help prevent ARs to other analgesics/NSAID in most patients with a history of adverse reactions to analgesics/NSAIDs.

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RUBBER PROTEINS AND MILK CASEIN AS ANTIGENS IN SURGEON'S GLOVES

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Surgeon's and other gloves made from natural rubber latex (NHL) are increasingly reported to cause immediate allergic symptoms ranging from contact urticaria to anaphylaxis. The risk groups are multioperated children and atopic health-care workers. To characterize allergenic proteins we performed immunoblot, crossed immunoelectrophoresis (CIE), crossed-line radioimmunoelectrophoresis (CLRIE) and immunospot (IS) studies in the eluates of 6 surgeon's gloves and NRL. Latex-allergic patient sera were used as a source of IgE and IgG4 antibodies. Total protein content was from 15 to 337 µg/g glove with Lowry method. IB showed from 0 to 7 antibody binding protein bands with mw ranging from 14 to 200 kD. The major antigens were 14, 30 and 39 kD proteins which were detected also in NRL. CLRIE confirmed the presence of several crossreacting antigens in the glove eluates and NRL. Immunospot, a modified dot-blot technique, showed that the intensity of IgE-binding varied from glove to glove brand. Interestingly, we could also identify with CIE cow's milk casein in some glove brands. This protein is added during glove manufacture and was shown to cause contact urticaria in one glove-using milk allergic subject. Therefore, in addition to natural rubber proteins casein should also be considered as a potential sensitizing agent in surgeon's gloves.

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SYSTEMIC MECHANISM OF PHOTOTHERAPY ON UREMIC PRURITUS.

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Uremic pruritus is poorly understood despite the high incidence among chronic renal failure (CRF) patients undergoing hemodialysis. Serum histamine levels have been demonstrated to elevate in CRF patients with itching and ultraviolet B (UVB) therapy, even if the irradiation on partial body, was reported to be beneficial for the generalized relief of pruritus. We have already demonstrated the local mechanism of UVB action; the ability of irradiation to suppress histamine release from mast cells. However, detailed systemic mechanism(s) remains obscure. Therefore, we aimed the below study. Sera of CRF patients with or without uremic pruritus were incubated with purified rat peritoneal mast cells and the obtained histamine release from mast cells was compared. Sera of uremic pruritus patients resulted in higher histamine release (44.60 ± 6.32 %, n=9, $p < 0.005$), than sera of patients without itching (19.71 ± 3.14 %, n=5, $p > 0.25$) and normal control sera (23.62 ± 7.14 %, n=6). This increased histamine release was dose-dependently restored to spontaneous release levels in five of seven patients by pre-exposure of the sera to UVB *in vitro*. From these results, sera of CRF patients with uremic pruritus was considered to contain some histamine releasing factor(s), which could be drained or diminished by UVB irradiation, suggesting a possible systemic mechanism of UVB action. We are now expanding to characterize the histamine releasing factor(s).

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EFFECTS OF ANTIALLERGIC AGENT ON CUTANEOUS PRURITUS IN HEMODIALYSIS PATIENTS WITH SPECIAL REFERENCE TO THE RELATIONSHIP OF PRURITUS TO PLASMA HISTAMINE CONCENTRATIONS

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Persistent pruritus is the most common symptom in hemodialysis(HD) patients. The causes of it are poorly understood and antihistaminic agents are not effective in many cases. Recently Marchi et al. reported that pruritus improved after the intravenous administration of recombinant human erythropoietin(EPO),a drug for renal anemia, with a decrease in plasma histamine concentrations(pHA)(N.Engl.J. Med. 1992;236:969-973). In order to determine whether pHA is related to pruritus in HD patients and whether azelastin hydrochloride(Azeptin),an antiallergic agent, would benefit pruritus with a pHA change, we measured pHA in maintenance HD patients using radioimmunoassay.

28 HD patients were divided into four groups according to the presence or absence of pruritus and EPO therapy. Regardless of pruritus or EPO therapy, the pHA of HD patients was significantly higher than healthy controls, but a significant difference could not be found among the four hemodialysis groups. The patients with pruritus showed a remarkable decrease in pruritus scores with azelastin hydrochloride treatment, but without pHA changes.

There seemed to be no correlation between pruritus scores and pHA. Antiallergic agents should be considered more appropriately as treatment for uremic pruritus.

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TREATMENT OF GENERALIZED SCLERODERMA WITH COMBINED TRADITIONAL CHINESE AND WESTERN MEDICINE REPORT OF 30 CASES. Wang Lei, Wang Dexin, Department of Dermatology, the Second Affiliated Hospital of Tianjin Medical College, Tianjin China

Generalized scleroderma is a disease of the skin connective tissue. Formerly, large doses of penicillin, EDTA, vasodilators such as nicotinic acid and immunosuppressors were used to treat this condition, but this treatment showed no manifest effects. In recent years, by treating these cases mainly with traditional Chinese drugs to promote blood circulation and end stasis, strengthen resistance and improve the nutritional status of the patient we have cured many patients.Most of the clinical symptoms can be alleviated or eliminated. Incases with serious general sysmptoms, we use Chinese drugs in combination with western medicine, chiefly corticosteroids, vasodilators and antibiotics. The results were satisfactory in all but 2 cases.

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STUDY OF CUTANEOUS MICROCIRCULATION AND APPROACH OF PATHOGENESIS IN RAYNAUD'S SYNDROME. Sen-An Yang, Gwo-Shing Chen, Shin-Su Yu, Department of Dermatology, Kaohsiung Medical College Kaohsiung, Republic of China.

Raynaud's syndrome (RS) is the paroxysmal constriction of small arteries of the extremities, usually precipitated by cold temperatures. The pathogenesis is not clear. This study hopes to clarify the mechanism and evaluate the prognosis and treatment of RS patients. Subjects are divided into three groups: (1) primary RS (PRS) patients (n=26) (2) secondary RS (SRS) patients (n=45), including progressive systemic sclerosis (PSS) patients (n=15), systemic lupus erythematousus (SLE) patients (n=20) and vibration white finger (VWF) patients (n=10) and (3) normal controls (n=25). We performed the following studies : (1) noninvasive microcirculatory assessment, including a. cold provocation test of skin temperature b. plethysmography c. laser Doppler flowmetry d. nailfold capillary morphology and blood cell velocity. The above examinations were measured at room temperature and after exposure to cold, respectively. (2) NaF dynamic capillaroscopy with dyes (3) hemorheologic studies, including a. anticardiolipin antibody (aCL) b. β -thromboglobulin (β -TG) c. von Willebrand factor (vWF) antigen d. 6-keto-prostaglandin F11 (PGF1 α) and (4) a sympathetic α receptor function test. The results are as follow: 1. Total finger blood flow significantly decreased in SRS patients and slightly

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A NOVEL MODEL OF SPONTANEOUS MIXED CONNECTIVE TISSUE DISEASE (MCTD)-LIKE SKIN LESIONS IN AGED MRL MICE. Fukumi Furukawa, Masahiro Takigawa, Takao Tachibana, Yuji Horiguchi, and Sadao Imamura, Department of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, and Faculty of Medicine, Kyoto University, Kyoto Japan

Epidermal nuclear stainings (ENS) by direct immunofluorescent (IF) methods are observed in the skin of mixed connective tissue disease (MCTD) and also systemic lupus erythematosus. The mechanism of ENS is still controversial. Recently, we found that aged MRL/Mp-+/+(MRL/n) mice showed the high incidence of ENS. Using this newly established model mouse, we examinded the possibility that this mouse will be a model for MCTD.

MRL/n mice were maintained under conventional conditions. Skin biopsy specimens were obtained from the back skin of 15 aged (18-20 mo) male, 18 young (1-9 mo) male, 15 aged (10-14 mo) female, and 13 young (1-9 mo) female mice. Direct IF studies revealed that 8 out of 15 aged male and 6 out of 15 aged female mice had ENS, but young male or female mice did not. ENS positive MRL/n mice showed also nuclear deposits of Ig in the kidney. Anti-RNP antibodies as well as anti-DNA antibodies were demonstrated by improved ELISA methods, but not by immunodiffusion methods. In vitro binding assay of antibodies to nucleus of PAM212 cells in culture showed the low incidence (4/30) binding to nucleus but there was no association between the binding and ENS positivity.

Aged MRL/n mice will give us a new clue for understanding the mechanism of ENS in skin specimens of collagen disease including MCTD.

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BORRELIA BURGDORFERI DNA IN MORPHOEA AND LICHEN SCLEROSIS ET ATROPHICUS: DETECTION BY THE POLYMERASE CHAIN REACTION. NJ Levell, KJ Cann[1], TA Leslie, ML Wilson[1], DJR Wright[1], Pauline M Dowd. Dermatology Department, University College London Medical School. [1]Medical Microbiology Department, Charing Cross and Westminster Medical School. London. UK.

Early studies have indicated serological evidence of Borrelia burgdorferi (BB) infection in certain patients with morphoea and lichen sclerosis et atrophicus (LS et A). This has been refuted in later studies which found negative serology. Detection of Borrelia flagella DNA using the polymerase chain reaction (PCR) on skin biopsied from a patient with morphoea following a tick bite has been reported and two recent studies of patients with LS et A have given conflicting reports of the presence of Borrelia DNA.

Four patients (3 male, 1 female; age 35 - 64) with morphoea and 1 female aged 54 with morphoea and vulval LS et A were investigated for BB infection using an enzyme linked immunosorbant assay (ELISA) and western immunoblotting on serum and a nested PCR technique on urine with primers specific for the BB outer surface protein A antigen.

The serological tests and PCR were negative in the 4 patients with morphoea. The patient with morphoea and LS et A had a positive PCR result and a weakly positive ELISA. Subsequent PCR of biopsied lesional skin from this patient was also positive. There was no history of tick bites. There was clinical improvement, but not clearance, of the vulval LS et A after 8 weeks treatment with oral doxycycline but little change in the cutaneous lesions of morphoea.

The detection of BB DNA from this patient indicates that BB infection may be involved in the pathogenesis of a subgroup of patients with morphoea and LS et A.

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HERPES SIMPLEX VIRUS DETECTION BY POLYMERASE CHAIN REACTION IN INTESTINAL ULCER OF PATIENTS WITH BEHÇET'S DISEASE. Eun-So Lee, Sungnack Lee, Dongsik Bang, Seonghyang Sohn, Chanil Park, Kyibeom Lee, Departments of Dermatology and Pathology, College of Medicine, Ajou University, Suwon, and Yonsei University College of Medicine, Seoul, Korea

Behcet's disease has been described as a chronic, recurrent, multisystemic inflammatory disease in which gastrointestinal involvement is occasionally seen. The etiopathogenesis of intestinal manifestation is not clear, but we have previously reported a possible etiopathogenic role of herpes simplex virus(HSV) DNA in the formation of oral ulceration in Behçet's disease. However, the clinical and morphologic characteristics of intestinal-Behçet's disease suggest a correlation with inflammatory bowel disease, making differential diagnosis very difficult. To demonstrate the possible role of HSV DNA in the pathogenesis of intestinal-Behçet's disease as well as Crohn's disease, we applied polymerase chain reaction (PCR) to detect HSV DNA from the lesions of both disease. We obtained seven paraffin-embedded tissue blocks from seven patients with intestinal ulcer who were diagnosed as Behçet's disease and thirteen blocks from patients with Crohn's disease. All specimens from patients with Behçet's disease showed a positive reaction to PCR, in contrast to only two positive reactions out of thirteen specimens from patients with Crohn's disease. It can be deduced that HSV could be considered one of the etiopathogenic factors in gastrointestinal lesions in Behçet's disease, and although differential diagnosis between Behçet's disease and Crohn's disease is difficult, detection of HSV DNA could contribute to the solution of the problem.

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BEHÇET`S DISEASE: TREATMENT WITH INTERFERON GAMMA

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Behçet`s disease is a chronic systemic inflammatory disease, its cause is unknown. Immunosuppressants may be effective treatments. We describe the therapeutic potentials of recombinant IFN-gamma. We admitted a total of 16 patients with Behçet`s disease in an open study with IFN-gamma. In the first two weeks we administered daily 50 - 100 µg recombinant IFN-gamma subcutaneously, thereafter injections were given three times a week. The study period was fixed at 6 months. After 6 months 13 of the 16 patients were evaluable. In one patient the therapy had to be discontinued after three months because of deterioration of the ocular symptoms. Three patients withdrew because of family affairs. The therapy was well tolerated, only mild influenza-like symptoms occured. Laboratory chemical analysis showed substance caused side effects such as reversible increase of monocytes, serum triglyceride concentration and lactat dehydrogenase activity. Under the long-term treatment the mucocutaneous symptoms became less pronounced or cleared completely. The ocular symptoms did not ameliorate. Some weeks after discontinuation of the treatment exacerbation of the disease appeared. The results suggest that IFN-gamma is a promising remedy for patients with arthritis and mucocutaneous lesions. The mode of action of IFN in Behçet`s disease remains unknown.

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ALOPECIA AREATA SERUM INHIBITS THE GROWTH OF NORMAL DERMAL PAPILLA CELLS. N.S. Calver, S.M. Parkin, [1]S. Macdonald Hull, [1]W.J. Cunliffe and V.A. Randall. Departments of Biomedical Sciences, University of Bradford, Bradford, BD7 1DP, UK and [1]Dermatology, The General Infirmary, Leeds, LS1 3EX, UK.

The aetiology of Alopecia Areata (AA) is unknown, although autoantibodies to hair follicle components have been found. Since the dermal papilla is believed to regulate the hair follicle, it would be a prime site for any factors inhibiting hair growth. Therefore, we have investigated the effect of serum from AA patients on cultured normal dermal papilla cells to determine whether it contains any cytotoxic factors.

Primary dermal papilla lines (n=5) were derived from normal scalp and their growth investigated in the presence of pooled normal and individual samples of AA serum. Cell growth was measured after 4 days in 96 well microtitre plates by the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) conversion assay and the results checked by haemocytometry.

There was a significant increase in growth of dermal papilla cells in the presence of foetal bovine serum or normal human serum validating the assay. Pooled AA serum inhibited growth significantly by 67%; alopecia totalis and alopecia universalis serum, when compared to normal serum, had no significant effect.

These results suggest that AA patient serum contains factor(s) which are cytotoxic to dermal papilla cells. These need further investigation to identify the specific components which may well be involved in the pathology of AA.

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AUTOANTIBODIES IN ALOPECIA AREATA. V.A. Randall, N.S. Calver, [1]S. Macdonald Hull, S.M. Parkin and [1]W.J. Cunliffe. Departments of Biomedical Sciences, University of Bradford, Bradford, UK and [1]Dermatology, The General Infirmary, Leeds, UK.

The aetiology of Alopecia Areata (AA) is unknown, although it is generally regarded as an auto immune disease. This study was designed to determine whether patients produce autoantibodies to components of the alopecic scalp or normal hair follicle.

Longitudinal frozen scalp sections from active edges of alopecic lesions and occipito-parietal region of normal controls were incubated with serum and/or anti-Human immunoglobulin fluorochrome conjugate.

No fluorescence, except that of the vasculature, was identified in normal scalp sections with normal serum. Alopecic scalp sections from 19 patients showed increased fluorescence from the outer root sheath in 78.9% of cases; subgroups showed fluorescence to other scalp keratinocyte structures. Fluorescence was also detected from incubation with the fluorochrome conjugate alone in 57.8%. Normal scalp sections incubated with alopecic serum showed similar immunofluorescence patterns, to patient sections.

These results demonstrate the presence of autoantibodies to normal hair follicular components, particularly the outer root sheath, in AA patients. They imply that AA autoantibodies react with normal structures and not abnormalities in alopecic follicles. The fluorescence obtained with the conjugate alone indicates that autoantibodies are deposited within the lesion prior to biopsy. Although these autoantibodies may be involved in the aetiology of AA, they could be produced as the result of damage to affected tissues.

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STUDIES ON THE PROLIFERATIVE RESPONSES OF LYMPHOCYTES TO CULTURED NORMAL MELANOCYTES IN VITILIGO PATENTS. Bian Zhao, Department of Dermatology, First Affiliated Hospital, Nanjing Medical College, Nanjing, P.R.China

So far the pathogenesis of vitiligo remains unclear. The dysregulation of cell–mediated immune reaction may contribute to the occurrence of vitiligo. In this study, the proliferative responses of lymphocytes to cultured normal human melanocytes (MC) in vitiligo patients were detemined. MC were cultured from the skin of normal individuals and pure MC were obtained after 7 to 10 days in subculture. The cultured cells exhibited typical MC morphology and displayed Dopa and anti–S 100 protein reactivities. Lymphocyte transformation test with cultured normal MC as specific antigen was perfromed in 26 vitiligo patients and 22 healthy individuals as control. It was found that the incorporation of ^{3}H TdR was 203.48 ± 28.94 cpm in the vitiligo patients, while 99.03 ± 12.15 cpm in the controls. The results indicated that the patients with vitiligo had a signficantly increased proliferative responses of lymphocytes to normal MC in comparison with the controls ($P < 0.01$). The results suggest that aberrations in cell–mediated immune reaction may be operative in the pathogenesis of vitiligo.

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APPLICATION OF EPILUMINESCENCE MICROSCOPY IN THE DIAGNOSIS OF PIGMENTED SKIN LESIONS IS A QUESTIONABLE TOOL FOR *NOT SPECIFICALLY TRAINED* DERMATOLOGISTS. Michael Binder, Margot Schwarz, Alexander Winkler, Andreas Steiner, Klaus Wolff and Hubert Pehamberger. Dept. of Dermatology Vienna, University of Vienna Medical School, Austria

Epiluminescence microscopy (ELM) is a non invasive clinical examination technique which, by employing the optical phenomenon of oil-immersion, makes subsurface structures of the skin accessible for in vivo examination and thus provides additional criteria for the clinical diagnosis of pigmented skin lesions (PSL). At present almost all studies about ELM are based on data derived from ELM-experts. In the present study we were interested in the question whether ELM indeed significantly improves the clinical diagnosis of PSL and whether specifically trained and not trained clinicians gain even information by the use of this technique. 240 photographic images of randomly selected histologically proven PSL, were presented with and without oil immersion. 6 ELM-experts and 13 ELM non-experts evaluated each PSL presented. To calculate the effects between oil-immersion technique and without and also between ELM-experts and non-experts the following parameters were obtained: Intra- and interobservers agreement by kappa statistics, sensitivity and specificity of diagnostic performance. ELM-experts exhibit a substantially better intraobservers agreement than non-experts (median kappa: 0.57 vs. 0.36), using oil immersion interobservers agreement was markedly increased in ELM-experts (average gain: 7%) but decreased in non-experts (average loss: 6%). Using oil-immersion sensitivity of diagnosis is significantly increased in ELM-experts (average gain: 10%) and decreased in non-experts. (average loss 10%) (2p=0.02). Specificity of diagnosis was excellent in ELM-experts with and without oil-immersion (92%) and was improved by oil-immersion in the non-expert group (76% vs 85%). Based on our data we conclude that the application of oil-immersion ELM significantly decreases the quality of the diagnostic performance of clinicians not specifically trained for ELM and thus the application of the ELM technique should be restricted to ELM experienced dermatologists.

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QUANTIFICATION OF NUCLEOLAR ORGANIZER REGIONS IN CUTANEOUS TUMORS. Sung Yul Lee, Chil Hwan Oh. Department of Dermatology, Korea University College of Medicine, Seoul, Korea.

The histologic differentiation between benign and potentially malignant conditions is oftendifficult. Staining ofnucleolar organizer regions(NORs) with a silver colloidal method were recently reported to give useful information on the benign or malignant potential of a given tumor. But many authors indicated a significant overlapping of NORs counts between benign and malignant proliferation. So we tried to investigate the various parameters including NORs counting in cutaneous tumors using image analysis system.(AIC.,Roswell) NORs were investigated on silver stained 4um section of 10 bowen dz, 16 squamous cell Ca, 16 seborreic keratosis, and 4 keratoacanthoma. For each case, 100 cell were examined using a x 100 oil immersion lens. There is no consensus at present as to the best criterion for quantifying NORs in cutaneous tumor. We prefer to quantify a large number of parameters. The mean value or standard deviation(SD), each calculated for a batch of 100 cells, are average again(mean±SD) for 5 parameters; mean numbers of NORs per nucleus, mean ratio of NORs area per nucleus area, mean area of largest NORs, mean ratio of largest NORs area per nucleus area, mean nucleus area per aNORs. We found significant differences in all 5 parameters in cutaneous tumors($p<0.01$). For example, mean ratio of NORs area per nucleus area were as follows; keratoacanthoma 0.11±0.02, seborreic keratosis 0.08± 0.02, squamous cell Ca 0.11±0.02, bowen dz 0.07±0.01. It is suggested that NORs analysis by image analysis system may be of use diagnosis of cutaneous tumors.

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ENGRAFTMENT OF LESIONAL SKIN OF HUMAN CUTANEOUS NEOPLASMS ONTO SCID (SEVERE COMBINED IMMUNODEFICIENCY) MICE. Yoshihiro Takizawa, [1] Yasutaka Tokuda, [1] Toshiaki Saida [1] and Yoshito Ueyama, [2] [1] Department of Dermatology, Shinshu University School of Medicine, Matsumoto; [2] 8th Laboratory, Kanagawa Academy of Science and Technology, Kawasaki; [2] Department of Pathology, School of Medicine, Tokai University, Isehara; [2] Central Institute for Experimental Animals, Kawasaki, Japan.

Using full-thickness skin grafting technique, total 19 lesions obtained from 11 cases of the following cutaneous neoplasms were transplanted onto the dorsum of C,B-17 SCID mice: solar keratosis(SK), thermal keratosis(TK), radiation keratosis(RK), Bowen's desease(BD), basal cell carcinoma(BCC) arising in nevus sebaceus, extramammary Paget's desease(PD), and radial growth component of acral lentiginous melanoma(MM). 18 of the 19 grafts were successfully accepted in the mice. Most grafts showed a slight tendency to contracture, however, substantial portion of each graft was maintained for a long period. 15 of the accepted 18 lesions were biopsied on 20 to 101 days after transplantation. These grafts were immunohistochemically positive with human blood group antigens ABH, confirming the successful acceptance of the human grafts. Neoplastic cells were histologically detected in 8 of the 15 grafts(1 of 1 graft of SK, 2 of 3 grafts of TK, 1 of 1 graft of RK, and 4 of 4 grafts of BD). In 2 grafts of PD and 3 grafts of MM, no definite neoplastic cells were detected, though the characteristic epidermal changes were preserved. BCC was not detected in the graft of nevus sebaceus. The present study suggested that engraftment of lesional skin could be used as a useful in vivo experimental model of human cutaneous neoplasms.

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HYPERTHERMIC TREATMENT OF BOWEN'S DISEASE.

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Bowen's disease represents a form of squamous cell carcinoma in situ. If hyperthermic treatment is indeed effective against cancer, this disease is thought to be also effective. We tried using local hyperthermic treatment with this disease. Three patients with biopsy-proven Bowen's disease were entered into the protocol. As a heat source, we used disposable chemical pocket warmers. They were secured directly against the lesion with a elastic bandage. The surface skin temperature was raised to 40-42 °C. The duration turned out to be 10 hours daily, while the patients were awake. The course of the disease was observed in two-weekly hospital visits by the patients. The clinical courses were marked by the following changes: the nodular lesions had become flattened and the erythematous and infiltrated lesions had shown a considerable reduction by the second or third week after the start of treatment, and by weeks 5 and 6, the lesions had become almost entirely pigmented. After three months of treatment, the lesions were excised, serial tissue sections were made from it and examined histopathologically. The examination revealed several cells judged to be atypical tumor cells remaining in case 1, and in case 2, an absence of tumor cells; but in case 3, no pathological tests were possible. In the dermatological field, there have been reports of the efficacy of hyperthermia in the treatment of cutaneous malignancies. However, until now, no papers have appeared on its use in cases of Bowen's disease. The results of the therapy were not perfect, but as a method of treating this disease, this technique is extremely promising.

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EXPRESSION OF HEAT SHOCK PROTEIN (HSP) 70, AND P53 SUPPRESSOR ONCOGENE PRODUCT IN LYMPHOMAS OF THE SKIN. Tetsuo Nagatani, Megumi Miyazawa, Toshiko Matsuzaki, Gaijiro Iemoto, Shutaku Kim, Naoko Baba and Hiroshi Nakajima, Department of Dermatology, Yokohama City University School of Medicine, Yokohama, Japan.

Stress proteins were initially identified as the major products of protein synthesis during the heat shock response, and have also been observed in response to a variety of different stress stimuli. Heat shock protein (hsp) 70 was recognized as a chaperone protein. By the way, mutation in the p53 tumor-suppressor gene was found at a high frequency in a wide variety of primary human cancers. In normal adult tissues synthesizing only wild type p53, the protein is difficult to detect, however mutant protein are degraded less rapidly than wild-type p53. Therefore, the mutant proteins accumulate to relatively high levels that can be detected readily in primary tumors by immunohistochemistry. Well, the mutant protein bind with high affinity to members of the hsp70 family of chaperon proteins, whereas wild-type p53 lack this type of association. Four cell lines, four biopsy samples from patients with ATLL, twenty-six biopsy samples from patients with CTCL were studied. Expression of hsp72/73 and p53 was extremely high in the culture cell lines, and also high in the samples from the patients with ATL, but low in the samples from the patients with CTCL. That is, co-expression of hsp72/73 and p53 was detected in some malignant cells and it seems that expression of HSP72/73 was dependent on not only physical stresses.

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SQUAMOUS CELL CARCINOMA AND BOWEN'S DISEASE IN JAPANESE HAWAIIANS. Tsu-Yi Chuang, George T. Reizner, and David J. Elpern. Department of Dermatology, Wright State University, Dayton, OH; Division of Dermatology, University of Wisconsin, Madison, WI; and Kauai Medical Group, Kauai, HI, USA.

Hawaiians are known to have the highest incidence of skin cancer in the U.S. But the skin cancer incidence in Japanese Hawaiians has seldom been reported. We thus investigate the incidence of squamous cell carcinoma(SCC) and Bowen's disease(BD) in Japanese Hawaiians for the period from 1983 through 1987. This is a prospective population-based incidence study conducted in resident Japanese in Kauai, Hawaii.

A total of 24 Japanese residents, 6 men and 18 women, were identified with an initial episode of SCC during the five year period. The annual incidence rate per 100,000 Kauai Japanese population was 22 for men and 67 for women with a combined rate of 44. The average patient age was 80 years. The head was the most common anatomic site with the limbs second. Subsequent new SCC and recurrent SCC after treatment are rare. Five patients had basal cell carcinoma simultaneously or at other time.

A total of 11 Japanese residents, 3 men and 8 women, were identified with new BD. The incidence rate per 100,000 Japanese Hawaiians was 11 for men and 30 for women with a combined rate of 20. The average patient age was 74 years. The limbs were the most common anatomic site. Subsequent new BD and recurrent BD occurred only occasionally.

Kauai's incidence rates of SCC and BD in Japanese population are much higher than documented in Japan. More Japanese women had SCC and BD than men. This peculiar finding was not observed in Japan. In comparison with the Caucasian Hawaiians, Japanese had SCC and BD at an older age. No consistent incidence trend is documented in the 5-year study.

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A NEW APPROACH FOR TREATMENT OF HYPERHIDROSIS USING PULSED CURRENT. Stephen Reinauer, Ansgar Neusser, Erhard Hölzle, Department of Dermatology, Heinrich-Heine-University, Düsseldorf, FRG.

The principle of tap water iontophoresis (TWI) using direct current (DC) is well established and represents the most effective treatment of palmoplantar hyperhidrosis. Side effects of this method are discomfort with burning and tingling, and skin irritation including erythema and vesicles. Uncontrolled use may induce iontophoretic burns and electric shock.

The aim of this study was to minimize side effects and to increase technical and safety standards of TWI without loss of efficacy.

In a controlled blind study two different new methods with alternating current (AC) were employed for treatment of palmar hyperhidrosis. The TWI-apparatuses were operated by a rechargeable energy source generating (I) AC of +/-8 V at a frequency of 5.1 kHz which produced a patient current of 8-12 mA rms or (II) a pulsed current from 0 to 16 V at a frequency of 10 kHz which produced a patient current of 8 mA DC with an AC current of 8-12 mA rms superimposed. (III) The conventional DC-method of TWI employing a maximum direct current of 25 mA and a maximum voltage of 54 V was used as a control.

The patients' palms were treated for 15 minutes once daily 4 times weekly. Sweat secretion rates were determined gravimetrically. Palmar hyperhidrosis was completely controlled in all patients (n = 10) after an average of 12 treatments using TWI with pulsed current and 10 treatments using the conventional DC-method (n = 10). Acrocyanosis and edematous swelling of fingers associated with hyperhidrosis subsided too. Virtually no effect was seen when AC without DC-offset was used for TWI up to 25 treatments (n = 10).

Efficacy of iontophoresis with pulsed current is virtually equal to the conventional DC-treatment. Plain AC has no effect. There were neither signs of cutaneous irritation nor sensations of subjective discomfort when AC or pulsed DC was employed. Mild electrical shock was prevented. Elaborated safety measures therefore are not necessary using pulsed current. Iontophoresis with a pulsed current should become the treatment of choice for palmoplantar hyperhidrosis.

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A CASE OF B-CELL CHRONIC LYMPHATIC LEUKEMIA ASSOCIATED WITH PRURIGINOUS SKIN LESIONS AND MARKEDLY ELEVATED SERUM IGE LEVELS: SUCCESFUL THERAPY WITH IFN α AFTER FAILURE OF IFNτ. **Johannes Ring[1], Barbara Berg-Drewniock[1], M. Neumaier[2], K. Becker[3], H. Zeller[3], Gerd Gross[1] and Karsten Neuber[1], Department of Dermatology (1), Department of Clinical Chemistry(2), Department of Hematology and Oncology (3), University Hospital Eppendorf, University of Hamburg, Germany**

A 54 year old male patient with a 5 year history of chronic pruritus and relapsing eczematous skin lesions as well as recurrent infections of the skin with *S.aureus* was investigated. Clinical evaluation showed disseminated papular erythematous partly excoriated skin lesions. Histologically, a superficial dermatitis was seen. The laboratory analysis revealed leukocytosis (15.9 x 10^9/l), neutropenia (32.9%), lymphocytosis (46.5%) and eosinophilia (11.9%) as well markedly elevated serum IgE levels (140,000 kU/l). Biopsy of the bone marrow showed a diffuse infiltrate of small lympho-plasmocytic lymphocytes. Over 80% of the lymphocytes were CD5+, CD19+, CD20+, CD23+, CD38+. Based on these findings the diagnosis of chronic lymphatic leukemia of the B-cell type was made. The strong pruritus was resistent to antihistamines and steroids. Therefore, a trial with daily 50 µg IFNτ s.c. was started in order to suppress elevated serum IgE. But the patients condition became worse. After 1.5 Mill. Units of IFNα s.c. every 2 days pruritus and serum IgE levels diminished markedly (< 50,000 kU/l). The patient is now in good condition for more then 6 months under continuing IFNα therapy. These findings argue for a possible beneficial effect of IFNα in suppressing IgE-production and symptoms of pruritus under certain clinical conditions.

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DEPICTION BY LORENZ CURVES AND QUANTIFICATION BY GINI COEFFICIENTS OF THE CONCENTRATION OF CASES IN DERMATOLOGIC DIAGNOSTIC CATEGORIES. Philip D. Shenefelt, Section of Dermatology, Department of Internal Medicine, University of South Florida, Tampa, Florida, U.S.A.

This study was designed to examine the distribution of skin diseases presenting at a clinic with a defined population base. All skin disease diagnoses were recorded by dermatologists at the University of Wisconsin (Madison) Student Health Center for three years from 1984 through 1986. Rank ordering of the diagnoses by frequency and graphing the cumulative percent of cases on the y-axis against the cumulative percent of the more than 200 diagnostic categories on the x-axis resulted in Lorenz curves. The further the curve deviates from the 45 degree slope line, the greater the concentration of cases in a limited number of diagnostic categories. Curves resulting from using data for first visits 1984-86, follow-up visits 1984-86, and total visits 1984-86 were similar and showed marked concentration of cases in relatively few diagnostic categories.

Calculating the Gini coefficient gives a quantitative expression of the ratio of the area between the curve and the 45 degree slope line to the total area above the 45 degree slope line. The Gini coefficient was 0.787 for first visits 1984-86, 0.845 for follow-up visits 1984-86, and 0.819 for total visits 1984-86. This reflects quantitatively the highly unequal distribution of cases among diagnostic categories.

The graphic and quantitative measures of concentration among diagnostic categories provided by the Lorenz curves and Gini coefficients can assist in determining a rational basis for resource allocation in health care planning for dermatologic diseases.

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CHRONIC ARSENICAL POISONING. Syed Atif Hasrat Kazmi, Umair Mansoor Bajwa, Syed Ghulam Shabbir, Department of Dermatology, Allama Iqbal Medical College and King Edward Medical College, Lahore Pakistan.

The study was carriedout to diagnose a mysterious disease in a village, manifesting as vague gastrintestinal complaints, weakness, dew drop hypo and hyper pigmentation of the skin and hyper keratosis of the palms and soles. The village was surveyed and investigated for suspected chronic arsenic poisoning. Tweny eight persons(16 males and 12 females) belonging to eight families were included because they had the above signs and symptoms. The hair and nails of the patients, the soil, fruits, vegetables and source of drinking water was tested for arsenic presence. Arsenic was present in the hair and nails of the affected individuals only and the source of drinking water consumed by them. Almost all the patients had low Hemoglobin concentration and raised Erythrocyte Sedimentation Rate(E.S.R.). All other laboratory investigations carried out were within normal range.

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SCANNING ELECTRON MICROSCOPIC AND X-RAY MICROANALYTIC STUDIES OF HAIR, NAIL AND FOLLICULAR KERATOTIC PAPULE IN PACHYONYCHIA CONGENITA. Naoto Ohtake, Hideaki Sugiyama, Katsuhiko Tsukamoto, Masutaka Furue and Kunihiko Tamaki, Department of Dermatology, Yamanashi Medical University, Yamanashi, Japan

A 2-year-old boy was diagnosed with Jadassohn-Lewandsky type of pachyonychia congenita (PC) because of nail hypertrophy, plantar blister and hyperkeratosis, follicular keratosis, oral leukokeratosis, and hoarseness. However, he had no sign of hair changes, neonatal teeth and steatocystoma multiplex.

Purpose: Surface structures and elements of the hair and nail in this patient were compared with those in normal boys. Morover, we investigated the structure and element of the follicular keratotic papule in comparison with those of the hair and nail in this patient.

Method: The hair, nail and follicular keratotic papule from this patient, and the hair and nails from 3 normal boys were studied with HITACHI S-2250N Natural SEM and HORIBA EMAX-5770 Energy Dispersive X-ray Micro Analyzar.

Results: The nail from this patient showed rougher surface structure and lower sulfur level than the nails from 3 normal boys. However, the hair from this patient showed the same surface structure and analytic pattern as the hair from 3 normal boys. The surface structure and analytic pattern of follicular keratotic papule with PC were much similar to those of his nail, but not those of his hair.

Conclusion: The hair of this patient was normal in scanning electron microscopic and X-ray microanalytic studies as well as clinical examination. As keratotic materials in nail hypertrophy and follicular keratosis showed similar low sulfur levels in X-ray microanalysis, we suggest that the decrease of the sulfur-containing amino acid is associated with hyperkeratosis in PC.

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DIAGNOSIS AND TREATMENT OF THE CUTANEOUS RADIATION SYNDROME: FOLLOW-UP ON CHERNOBYL ACCIDENT VICTIMS. Ralf U. Peter*,**, Andrei Birioukov*, Walter Kaffenberger**, Otto Braun-Falco*, Gertraud Krähn*, Martina Kerscher*, Ursula Peterseim*, Birger Konz*, Manfred Bauchinger***, and Gerd Plewig*; *Department of Dermatology, Ludwig-Maximilians-University, **Institute of Radiobiology, FAF Medical Academy, Munich, Institute of Cytogenetics, Gesellschaft für Strahlen-und Umwelfforschung, Neuherberg, Germany

On April 26, 1986, the most severe accident in the history of civilian use of nuclear energy in Chernobyl, Ukraine, led to acute overexposure of more than 200 people, causing death due to acute radiation disease and associated symptoms in 32 cases. Of these, 14 died from cutaneous lesions caused primarily by beta irradiation, involving < 40% of the body surface. Since September 1991, fifteen survivors of the accident, presenting with extensive cutaneous radiation-induced lesions have been exmined, treated and regularly followed-up at our department. The clinical picture was determined by dryness of the involved skin, teleangiectasias, keratoses, and partly severe subcutaneous fibrosis. Radiation ulcers were present in five patients. No cutaneous or internal malignancies could be detected. Patients suffered from recalcitrant herpesvirus infections. Antibodies against hepatitis B or C were noted in 9 patients. Bone marrow doses ranged from 0,7 to 5,8 Gy in 13 cases. Hydrating ointments and emollients led to marked improvement of involved areas. Aromatic retinoids, given orally in 2 cases (Etretinate, 0,2-0,5mg/kg bodyweight), and applied topically in 3 cases (Tretinoin, 0,05 %), lead to a reduction of radiation keratoses in the involved areas. Irritation caused by tretinoin could be antagonized by alternating topical application of nonatrophogenic steroid creams (Mometasonefuroate). Treatment of seven patients with low-dose gamma-interferon (Polyferon^{R}, 50µg 3x/week) for 6-18 months resulted in decreased density of fibrotic plaques, as determined by cutaneous sonography. Additionally, flow cytometric analysis revealed an accelerated phagocytotic capacity of granulocytes and monocytes, compared with untreated controls.
Surgery was performed in five patients for excision of ulcers, fibrotic plaques, radiation keratoses, correction of contractures. Primary wound closure, split skin grafts, pediculated and vascularized flaps were performed without complications.

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"Wash leather scrotum" (scrotal dermatitis): a treatable cause of male infertility

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The scrotum has a thermoregulatory function that is essential for normal spermatogenesis, and there is ample evidence both in animal and in man that elevation of testicular temperature leads to impairment of function. It has been known for many years that thickening of the scrotal skin because of elephantiasis is associated with testicular atrophy, and there is also evidence that eczema of the skin of the scrotum, or local inflammation and infection, has an adverse effect on seminal quality in rams. We have found evidence of a similar condition associated with infertility in 16 human males, and report a simple and effective treatment for the dermatological disease which restored fertility in one third of cases. The effect on sperm count and motility has been documented before and after treatment and their relationship to pregnancy in the female partner.

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TOPICAL RETINOIC ACID FOR HYPERPIGMENTED LESIONS ASSOCIATED WITH PHOTOAGING IN JAPANESE AND CHINESE. CEM Griffiths, MT Goldfarb, LJ Finkel, CN Ellis, JJ Voorhees. Department of Dermatology, University of Michigan, Ann Arbor, MI, USA

Hyperpigmented lesions are the predominant component (commoner than wrinkling) of photoaging in Japanese and Chinese patients and are a frequent source of concern. Topical all-*trans* retinoic acid (RA) cream improves hyperpigmentation associated with photoaging in Caucasians. We performed a study to assess the efficacy of 0.1% RA cream in the treatment of hyperpigmented lesions associated with photoaging in Japanese and Chinese patients.

Forty-five, photoaged patients (22 Japanese, 23 Chinese, mean age 60 years) completed a double-blind, randomized study in which 21 applied 0.1% tretinoin cream and 24 vehicle cream once daily to face and/or hands for 40 weeks. Patients' hyperpigmented lesions were evaluated clinically and by colorimetry throughout the study and by histologic analysis of skin biopsies taken at pretherapy and end of treatment.

At end of treatment, hyperpigmented lesions of the face and hands were lighter or much lighter in 90% of patients receiving RA compared to 33% receiving vehicle ($P < 0.0001$). Significant lightening occurred after only 8 weeks RA treatment ($P < 0.05$). There was no difference between Japanese and Chinese patients in their response to RA treatment. Colorimetry, an objective measure of skin color, demonstrated significant lightening of lesions after RA compared to vehicle ($P < 0.05$). Colorimetric lightening correlated with clinical improvement ($r = 0.54$, $P = 0.0001$). Histologic analysis of hyperpigmented lesions, 70% of which were actinic lentigines, demonstrated a significant 41% decrease in epidermal pigmentation with RA therapy as compared with a 37% increase in the vehicle group ($P = 0.0004$). Decrease in histologic epidermal pigment correlated with clinical response ($r = 0.45$, $P = 0.008$). No patient withdrew for adverse effects.

By clinical, colorimeter, and histological evaluation, 0.1% RA cream significantly and safely lightens the hyperpigmentation associated with photoaging of Japanese and Chinese skin.

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ENDOGENOUS EGF-FAMILY GROWTH FACTORS ARE NECESSARY FOR TRANSITION FROM G1 TO S PHASE IN HUMAN KERATINOCYTES, Teruaki Kobayashi, Koji Hashimoto, Hidenobu Okumura, Hideo Asada, Kunihiko Yoshikawa, Department of Dermatology, Osaka University School of Medicine, Osaka, Japan

It has been shown that EGF is necessary for advancing from G1 to S phase in fibroblasts. However, there have been no established reports on roles of EGF or EGF-like growth factors in cell cycle transition from G1 to S phase in human keratinocytes. Recent studies showed that human keratinocytes produce endogenous EGF-like growth factors such as TGF-α and amphiregulin. We hypothesized that these endogenous EGF-like growth factors are key factors in advancing from G1 to S phase in human keratinocytes. Human keratinocytes were cultured in serum-free MCDB 153 medium and then synchronized under amino acid deficiency condition. Cell cycle were analyzed by flow cytometry. The number of S phase cells increased to 35.2% at optimum 21 h after replating. However, addition of anti-EGF receptor blocking antibody (1 µg/ml) at 3 h after replating decreased S phase cells to 26.5%. In contrast, no decrease of S phase cells were observed by addition at 12 h or later. Northern blot analysis demonstrated 2 fold increase of TGF-α mRNA and 3 fold increase of amphiregulin mRNA at 0.5 h, followed by gradual decrease. These results indicate that endogenous EGF-like growth factors are involved in early phase of G1 to S phase transition in human keratinocytes.

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IMMUNOCHEMICAL LOCALIZATION OF HB-EGF, A NEW MEMBER OF EGF FAMILY, IN NORMAL SKIN, Mari Higashiyama,[1] Koji Hashimoto,[1] Shigeki Higashiyama[2] and Kunihiko Yoshikawa,[1] [1]Department of Dermatology and [2]Department of Biochemistry, Osaka University School of Medicine, Osaka, Japan

Heparin-binding EGF-like growth factor (HB-EGF) is a new member of EGF family originally purified from conditioned medium of the U-937 macrophage-like cell line. Recently, we found that HB-EGF was an autocrine growth factor for human keratinocytes. Namely, HB-EGF stimulate human keratinocyte growth. And, the presence of HB-EGF mRNA is detected in human keratinocytes. Furthermore, Western blot analysis confirmed the production and secretion of HB-EGF by human keratinocytes. In this study, we investigated the localization of HB-EGF in normal human skin. The rabbit anti-HB-EGF antibody was raised against synthetic peptide of amino acid residues 144 to 160 of HB-EGF. The avidin-biotin peroxidase technique was used for detection of HB-EGF using this antibody. HB-EGF was detected as granular or membranous staining in the intercellular space of the whole epidermis. However, the staining is more intense in the lower epidermis. Basal cells were stained most markedly. It should be noted that endothelial cells in the dermis also demonstrated the strong staining of HB-EGF. These data indicate that HB-EGF play important roles in growth regulation of human epidermis in vivo.

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STIMULATION OF FIBRONECTIN EXPRESSION BY AUTOCRINE TRANSFORMING GROWTH FACTOR-β IN CO-CULTURE OF KERATINOCYTES AND FIBROBLASTS. Chiyo Sato, Ryoji Tsuboi and Hideoki Ogawa Department of Dermatology, Juntendo University School of Medicine, Tokyo, Japan.

Synthesis of extracellular matrix components is an important event during the wound healing process. Several growth factors have been known as stimulants of wound repair, but only transforming growth factor-β (TGF-β) stimulates synthesis of extracellular matrix components. We examined the expression of fibronectin in co-culture of keratinocytes and fibroblasts. Various concentrations of secondary cells were seeded on the monolayer of primary cells and cultured in serum-free condition. Co-culture performed fibroblasts as primary cells significantly increased mRNA level of fibronectin when compared with that induced by mono-culture. Neutralizing antibody against TGF-β abolished mRNA expression of fibronectin induced by co-culture. Western blotting and immunoprecipitation revealed that protein levels of fibronectin also increased in co-culture and decreased by additional treatment with anti-TGF-β antibody. These results suggest that fibronectin synthesis is stimulated by direct contact between fibroblasts and keratinocytes through the activation of autocrine TGF-β.

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EFFECTS OF TRANSFORMING GROWTH FACTOR BETA, GAMMA-INTERFERON AND HISTAMINE ON CULTURED KELOID FIBROBLASTS. Kanako Kikuchi, Takafumi Kadono, Kazuhiko Takehara and Yasumasa Ishibashi, Department of Dermatology, Tokyo University, Faculty of Medicine, Tokyo, Japan

Wound healing in susceptible individuals could result in the development of keloids with over-abundance of extracellular matrix components. Although the disease pathogenesis is still unknown, growth factors or mast cell products such as histamine may be involved in the process of keloid formation. In this study, we investigated the effects of several growth factors on growth and collagen metabolism in keloid fibroblasts and control fibroblasts. Five fibroblast cell strains each derived from keloid or normal skin exhibited similar response to platelet-derived growth factor(PDGF), epidermal growth factor(EGF), transforming growth factor β (TGF β), g-interferon(γ-IFN) or histamine on DNA synthesis. On the other hand, procollagen type I carboxyterminal propeptide(P1CP) production, which reflects the amount of newly synthesized type I collagen, was fivetimes higher in keloid fibroblasts than in controls. TGF β(1ng/ml) treatment upregulated P1CP production in both cell strains. Similarly, treatment with histamine increased P1CP production 2.4 times in keloid fibroblasts, although it did not change tnat in control fibroblasts. Additionally, γ-IFN treatment decreased the P1CP production in both cell strains and the effect was greater in keloid fibroblasts. These data suggest that these factors are potent modulators of collagen metabolism in keloid fibroblasts and play a partial role in their pathogenesis.

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INSULIN-LIKE GROWTH FACTOR-I(IGF-I) AND INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-1(BP-1) PROMOTE WOUND HEALING IN DB/DB MICE. Chong-Ming Shi, Ryoji Tsuboi and Hideoki Ogawa, Department of Dermatology, Juntendo University School of Medicine, Tokyo, Japan

The synergistic effect of IGF-1 and BP-1 in promoting wound healing was examined in diabetic C57BL/KsJ *db/db* mice. Each mouse had two 6 mm diameter full-thickness wounds on the center of its back. Various concentrations of IGF-1 and/or BP-1 dissolved in 0.1% BSA-PBS were applied locally to the open wound once a day for 5 days. All mice were sacrificed on the 8th day and histological sections were evaluated using three parameters. Reepithelialization and areas of granulation were measured by IBAS computerized morphometric analysis, and the number of capillary lumens was counted at 100X magnification. Initially, the combination effect of IGF-I and BP-1 was examined. Wound healing, as measured by three parameters, was accelerated by each of the treatments in descending order of IGF-I plus BP-1, IGF-I, BP-1, vehicle alone. Interestingly, BP-1 alone showed mild stimulatory activity in spite of its lack of mitogenicity *in vitro*. With increases in concentrations in either IGF-I from 1 to 50 μg/ml or in BP-1 from 1.6 to 165 μg/ml, wound repair was accelerated. Maximal effect in all the three parameters was achieved by application of a combination of IGF-1(50μg) and BP-1(165μg) to each wound site. A five day application was found to be more effective in terms of wound repair than that achieved by a single application on the day operated. These results demonstrate that IGF-I and BP-1 synergistically promote wound healing in diabetic mice and further suggest the possible clinical use of IGF-I and BP-1 as a stimulant for wound healing.

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HEPATOCYTE GROWTH FACTOR STIMULATES HAIR GROWTH OF MOUSE VIBRISSAE IN ORGAN CULTURE. Toshimasa Jindo, Ryoji Tsuboi, Ryusuke Imai, Kenji Takamori, Jeffrey S. Rubin*, and Hideoki Ogawa, Department of Dermatology, Juntendo Univ. School of Medicine, Tokyo, Japan, *Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, MD, USA.

Hepatocyte growth factor (HGF) is a multifunctional polypeptide which works as mitogen, motogen and morphogen. In this study, we examined the effect of HGF on hair growth using an organ culture system. Vibrissal hair follicles isolated from new born mice were cultured at 31°C in 95% O_2-5%CO_2 for 72 hr in the presence of various cytokines and growth factors. The elongation of the hair shaft, DNA and protein synthesis in hair follicles were then measured. Among the tested materials, only HGF at 10 ng / ml significantly increased hair growth ($p<0.001$), ^{3}H-thymidine ($p<0.001$) and ^{35}S-cysteine ($p<0.05$) uptake. The effect of HGF increased dose dependently up to 10 ng / ml, and the addition of a neutralizing antibody against HGF canceled HGF-stimulated hair growth and DNA synthesis. These results indicate that HGF is a potent stimulator of hair growth and that it has a possibility to be used as a hair growth stimulator for clinical use.

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PHENOTYPIC MODULATION OF CULTURED ENDOTHELIAL CELLS IN COLLAGEN MATRICES INDUCED BY TUMOR NECROSIS FACTOR ALPHA. Shuzo Nakatani, Lu Lu, Natsuko Okada and Kunihiko Yoshikawa, Department of Dermatology, Osaka University School of Medicine, Osaka, Japan

The effect of TNF, a cytokine mainly produced by activated macrophages, on vascular endothelial cells was analyzed using a collagen-embedded three dimensional culture system, focusing on angiogenesis and expression of cell adhesion molecules. When the endothelial cells were cultured between two layers of type-1 collagen gel, they reorganized into a network of branching and anastomosing tubular structures. Once the structure was formed the cells did not undergo further division. Addition of TNF at 10-500 U/ml to the overlaid culture medium inhibited the tube forming process, and prolonged their survival time. To test the effect of TNF on the expression of cell adhesion molecules of the endothelial cells grown in collagen matrix, the collagen was digested with collagenase, and the dispersed cells were stained with anti ICAM-1 and ELAM-1 monoclonal antibodies. By FACScan analysis, both ICAM-1 and ELAM-1 expression in these cells were induced and accelerated in a dose dependent manner. Even in the absence of TNF, the cells expressed small amounts of these adhesion molecules. These results indicate that endothelial cells display phenotypic changes in collagen matrices, and modulatory response to TNF.

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FIBROBLAST FACTORS INDUCE MATURATION OF IMMATURE HUMAN MAST CELLS. Juergen Grabbe, Edgar Dippel, Pia Welker, Frank Schmidt and Beate M. Czarnetzki, Department of Dermatology, Rudolf Virchow Clinics, Freie Universität, Berlin, Germany

Mast cells are known to derive from hematopoietic progenitors and to differentiate in the tissue under the influence of fibroblasts. Stem cell factor, other fibroblast products and additional serum derived mediators are implicated in this process. In the present study, we have investigated whether such factors also play a role in late differention steps of immature mast cells. Cells of the human mast cell line HMC-1 were therefore cultured with horse serum and murine fibroblast supernatants for up to 10 days. At days 0 and 10, cellular contents and IgE mediated release of histamine and tryptase activity were determined spectrofluorometrically. Expression of the $Fc_\epsilon RI$ was analysed by FACS with a monoclonal antibody to the receptor α-chain and by Northern blotting. Under the culture conditions, intracellular histamine and tryptase activity increased twofold. This was paralleled by an increase of stimulated release of mediators. In addition, a marked upregulation of high affinity binding sites for IgE was noted and confirmed by an increase of mRNA for the $Fc_\epsilon RI$ α-chain. Besides early mast cell differentiation from hematopoietic progenitors, fibroblasts may thus also play a role in maturation of mast cells and the regulation of their functional state in allergic reactions.

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PRODUCTION OF A NOVEL FACTOR INDUCING FIBROBLAST-DEPENDENT MAST CELL GROWTH BY MURINE KERATINOCYTE-DERIVED SQUAMOUS CELL CARCINOMA CELLS. Toshihiko Tanaka, Koji Nakamura, Eishin Morita, Shoso Yamamoto, Department of Dermatology, Hiroshima University School of Medicine, Hiroshima, Japan

An increase in the number of mast cells has been observed at the sites of cancer. In order to know the mechanism by which mast cells increase at cancer sites, we investigated whether cancer cells produce some factor to induce mast cell growth by using a murine keratinocyte-derived squamous cell carcinoma cell line (KCMH-1). Although no activity of previously-known mast cell growth factors (such as IL-3, IL-4, IL-9, IL-10, soluble SCF or NGF) was detected in conditioned medium (TCM) of the KCMH-1 cells, marked enhancement of mast cell growth was observed when bone marrow-derived mast cells obtained from $WBB6F_1 \rightarrow +/+$ mice were cultured on NIH/3T3 fibroblast monolayer in the presence of TCM. 3T3 fibroblasts pretreated with TCM enhance mast cell growth even in the absence of TCM during the coculture. Although SCF and NGF are fibroblast-derived factors for mast cell growth, the results of Northern blot analysis showed no enhancement of levels of mRNA expression for SCF or NGF in TCM-treated 3T3 fibroblasts. Furthermore, in a mast cell-fibroblast coculture system, TCM also enhanced growth of W/W^v mast cells which lack the c-kit receptor. These results suggest that KCMH-1 cells may produce a factor which support mast cell growth synergistically with 3T3 fibroblasts, or activate 3T3 fibroblasts to grow mast cells by a novel mechanism different from that previously known.

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OCCURRENCE AND DISTRIBUTION OF PEPTIDERGIC NERVE FIBERS IN RAT SKIN DURING WOUND HEALING. Joanna Wallengren (1), Rolf Håkanson (2), Frank Sundler (3), Departments of Occupational Dermatology (1), Pharmacology (2) and Medical Cell Research (3), Lund University, Lund, Sweden.

Several neuropeptides may play a role as trophic factors. The healing of wounds induced by 3 mm punch biopsies on the medial aspect of the upper legs of female rats was studied. Specimens from the area around the wounds were taken on the same day and after 1,2,3,4,6,7 and 14 days. The distribution of nerve fibers was studied by immunohistochemistry using antibodies to substance P (SP), calcitonin gene-related peptide (CGRP), neuropeptide Y (NPY), vasoactive intestinal peptide (VIP) and a pan-neuronal marker (PGP 9.5).

One day after producing the wound small vessels and hair follicles appeared at the bottom of the wound; they had proliferated on the 2nd day. The density of SP-and CGRP-IR (immunoreactive) nerve fibers was low in the wound area. On the 3rd day a thin epithelial layer was seen to cover the wound. SP-and CGRP-nerve fibers proliferated at the edge of the wound. The epithelial layer became progressively thicker on the 3rd to 7th day. The hair follicles became enlarged on day 6-7. SP-and CGRP-IR nerve fibers proliferated in the wound area. On the 14th day there was a scar formation with few vessels and hair follicles. The density of SP- and CGRP-IR nerve fibers was lower than in control skin. NPY-and VIP-IR nerve fibers were rare in both control and in wounded skin. PGP-IR nerve fibers did not display any variation during the healing process.

The appearence of SP-and CGRP-IR nerve fibers at the edge of the wound on the 3rd day was followed by epithelialization. The proliferation of SP-and CGRP-IR fibers in the wound area on day 6 was followed by proliferation of hair follicles.

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CsA AND FK 506 EXERT DIFFERENT EFFECTS ON THE PHOSPHORYLATION OF TYROSINE RESIDUES IN PSORIATIC LYMPHOCYTES. H.M. Ockenfels, T. Schultewolter, P.M. Burger and M. Goos; Dept. Dermatology, University Essen, FRG.

Protein tyrosine kinases (PTK) are among the molecules that have been implicated to control cell growth and differentiation. One of the first steps in the activation of T-lymphocytes following the binding of antigen, the binding of monoclonal antibodies to the antigen-receptor complex or to other cell surface structures consists of the phosphorylation of proteins at their tyrosine residues. Psoriasis is discussed to be an immunological disease, i.e. that T-lymphocytes (T-lc) play a prominent role in pathogenesis of psoriasis. Immunosuppressive agents which inhibit IL-2 secretion, like cyclosporine A or FK506, are employed for therapy. We examined the time course of phosphorylated tyrosines as a marker for cellular tyrosine kinase activity in T-lc from psoriatics and controls after stimulation with phytohemagglutinin (PHA) and CsA or FK506. T-lc from controls show peaks of phosphotyrosine (p-tyr) after 15 min and after 4 h of PHA stimulation; in T-lc from psoriatics the 15 min peak is smaller, whereas the 4 h peak is significantly higher than after 15 min. Additional treatment of T-lc with CsA reduces the 15-min and 4-h p-tyr amounts more in psoriatics, i.e. to almost 60%, than in controls. By contrast FK506 did not alter the p-tyr amounts in controls or in psoriatics significantly after 15 min. After 4 hours FK506 diminished p-tyr levels to 70% in controls only. These data indicate, first, an important impact of T-lc activated via tyrosine-phosphorylated proteins on the pathogenesis of psoriasis and, second, differing antipsoriatic mechanisms of the two drugs FK506 and CsA.

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RAS TRANSFECTION ALTERS IP_3/ CA^{2+} SIGNALLING IN HUMAN KERATINOCYTES. Biao Shi, and R. Rivkah Isseroff, Dept. of Dermatology, University of California, Davis, Davis, CA .

Cultured keratinocytes respond to increases in the Ca^{2+} concentration in the medium by acquiring the differentiated phenotype; this is prevented by *ras*-transfection. To determine how *ras*-transfection perturbs the transduction of the extracellular Ca^{2+} (Ca_o^{2+}) signal in keratinocytes, we examined responses of inositol phosphates and intracellular Ca^{2+} (Ca_i^{2+}) to Ca_o^{2+} in a *ras*-transfected line I-7 and its non-transfected parental line HaCaT. Switch of Ca_o^{2+} from 0.05 mM to 1.5 mM induced a 1.5-2 fold increase in IP_3 release in HaCaT, with little effect on IP_3 level in I-7. The downstream pathway for IP_3 generation appears intact in the I-7 line, since either the receptor agonist bradykinin or the G protein activator GTP[S] induce equivalent increases in IP_3 in both HacaT and I-7 cells. Fluorimetric measurement of fura-2/AM-labeled monolayer keratinocytes demonstrated that stimulation of 1.0 mM Ca_o^{2+} induced a similar Ca_i^{2+} increase in HaCaT and I-7 cells: a initial transient rise (5-8 fold in HaCaT, 9 -15 fold in I-7 cells above basal level) followed by a sustained lower plateau. However, the Ca_i^{2+} plateau was maintained for 24 hours in HaCaT cells, while in I-7 cells, the Ca_i^{2+} concentration fell to near baseline level over this time period. Furthermore, stimulation of I-7 monolayer cells with 0.25 - 0.5 mM Ca_o^{2+} induced an intracellular Ca^{2+} oscillation (frequency of spike/4 - 5 min, magnitude of 40 - 100 nM) lasting >1 hour, not seen in HaCaT cells. These differences in the response to Ca_o^{2+} indicate that *ras* transfection alters the Ca^{2+} sensing mechanism and subsequent downstream transduction system in keratinocytes.

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EVIDENCE FOR THE OCCURRENCE OF Na-K-2Cl COTRANSPORTERS IN ECCRINE CLEAR CELLS AND THEIR REGULATION BY PROTEIN KINASE A AND THEIR INHIBITION BY PROTEIN KINASE C. T. Toyomoto, D. Knutsen, M. Ohtsuyama, F. Sato, S. Cavallin, and K. Sato, Marshall Dermatological Research Laboratories, University of Iowa College of Medicine, Iowa City, Iowa, USA.

The pharmacological basis for the regulation of ion transport during sweat secretion is still poorly understood, particularly the mechanism for regulation of Na-K-2Cl cotransporters. Although the presence of Na-K-2Cl cotransporters has been predicted in the sweat clear cells, direct evidence has been lacking. It has been puzzling how the drastic cell shrinkage after cholinergic (ACh) stimulation can be mitigated for sustained sweat secretion. Na-K-2Cl cotransporters are involved in cell volume regulation and thus their regulation can be studied by determining cell volume regulation. Using the image analysis method and collagenase-dissociated eccrine clear cells, we observed that 0.1 mM bumetanide inhibited pseudo-regulatory volume increase (PRVI, recovery of cell volume from cell shrinkage which occurs when the medium is switched from hypotonic to isotonic), partial cell volume recovery during MCh-induced cell shrinkage (VRPMS), and regulatory cell volume increase during hyperosmotic shock (RVI), suggesting that cotransporters are involved in cell volume recovery following various osmotic perturbations. Stimulation of cellular cAMP by 10 μM forskolin + isoproterenol significantly enhanced bumetanide-sensitive cell volume recovery during PRVI, VRPMS, and RVI. In contrast, the protein kinase C (PKC) activators, phorbol ester (TPA), and high concentrations of methacholine inhibited constitutive and cAMP-stimulated cell volume recovery. Okadaic acid (at 1 μM), which stimulates protein phosphorylation, stimulated RVI. We postulate that the Na-K-2Cl cotransporters which are involved in cell volume regulation are also involved in sweat secretion and that the cotransporters are stimulated by protein phosphorylation by PKA but inhibited by PKC. Thus the periglandular adrenergic innervation is instrumental in activating the cotransporters and alleviating the potential deleterious effect of acetylcholine-induced drastic cell shrinkage.

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THE PRESENCE OF CIRCULATING PEMPHIGUS VULGARIS ANTIBODIES (PV-IgG) IN FIRST DEGREE RELEATIVES OF JEWISH PEMPHIGUS PATIENTS
S. Yorav[1], A. Feinstein[1], D. Elstein[2], M. Schewach-Millet[1], B. Michel[3], E. Lyubimov[2] and Y. Milner[2] [1]Dept. of Dermatoly, Sheba Medical Center, Tel-Hashomer, Israel [2]Myres Skin Biochemistry Unit, The Hebrew University, Jerusalem, Israel. [3]CPI Laboratory Beachwood, Ohio, USA.

Past serological evidence indicated a genetic linkage of pemphigus in the Jewish population. Recently molecular biological studies showed that two alleles of the class II HL-AD hypervariable region actually confer to individuals the pemphigus susceptibility. In order to confirm the genetic disposition and to find if actually the pemphigus gene is expressed in vivo, we have analysed sera from 23 pemphigus patients and 58 of their first degree healthy relatives (FDHR) along with 53 normal sera. We have used the indirect immunoflourescence and ELISA method on cultured carcinoma cells for titer determination. The control sera showed average titers of 1:5 but some 0 type sera (≈10%) showed titers of 1:20. In the patients group (21-PV, 1-PE and 1-PF) 18 (78.2%) had positive pemphigus titers of above 1:20 (some of them as high as 1:640-1:1280). In the FDHR group 25 (43%!) showed titers of above 1:20 (some as high as 1:320-1:640 but the majority low to intermediate titers: 1:80-1:160). 90% of the FDHR PV-positive sera immunoreacted with the PV antigen (130 kDa polypeptide) and plakoglobin (85 kDa) in Western blots of SDS-PAGE separated epidermal and carcinoma membrane SDS extracts. Thus PV positive FDHR sera contains true pemphigus circulating antibodies. These findings show that the PV susceptibility genes are transferred and expressed in a significant proportion of inflicted families.

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IMMUNOGENETIC ANALYSES IN JAPANESE PEMPHIGUS VULGARIS. Hironori Niizeki[1, 3], Hidetoshi Inoko[2], Nobuko Inamoto[1], Takashi Hashimoto[3] and Takeji Nishikawa[3], [1]Section of Dermatology, Kitasato Institute Hospital, Tokyo, Japan, [2]Department of Molecular Life Science, Tokai University School of Medicine, Kanagawa, Japan, [3]Department of Dermatology, Keio University School of Medicine, Tokyo, Japan

We have previously studied HLA-DQA1 and -DQB1 genotyping in Japanese pemphigus vulgaris (JPV) patients, and found that DQA1*0103 and DQB1*0601 were absent and the frequency of DQB1*0503 was significantly increased compared with healthy individuals (corrected $p<0.0005$). About 60% of JPV patients carried this susceptible allele, DQB1*0503, but we didn't find any specific alleles within DQA1 and DQB1 loci in 40% of JPV patients. Therefore we performed DRB1 genotyping in 20 JPV patients by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The exon 2 domain of the DRB1 gene, which is highly polymorphic, was amplified by PCR, digested with 12 endonucleases. They were subjected to electrophoresis in 12% polyacrylamide gels and then the digested fragments were detected by staining with ethidium bromide. Forty five DRB1 genotypes were defined by cleavage and RFLP patterns. DRB1*0402, which is strongly associated with Jewish PV, was absent. The frequency of DRB1*1405, 1406 and 0406 were increased compared with controls ($p<0.000001$, 0.00005, 0.05). DRB1*1406 is a rare allele among Japanese population, and it is associated with DQB1*0301, but not 0503. Moreover, 17/20 patients carried the DR14-associated alleles (DRB1*1401, 1405 and 1406), and the rest of the patients (3/20) carried DRB1*0406. In summary we detected three haplotypes in 20 JPV patients; 1) DQ5-associated haplotype (DRB1*1401 or 1405-DQA1*0101 or 0102-DQB1*0502 or 0503), 2) DRB1*1406-DQA1*0501-DQB1*0301, 3) DRB1*0406-DQA1*0301-DQB1*0302.

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PEMPHIGUS VULGARIS AND PEMPHIGUS FOLIACEUS.
A STUDY OF 19 CASES BY IMMUNOELECTRONMICROSCOPY

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Pemphigus vulgaris (PV) and pemphigus foliaceus (PF) are autoimmune blistering diseases characterized by a loss of cell-cell adhesion and by autoantibodies directed against epidermal cadherins. PF antigen has been established to be desmoglein which is strictly located to the desmosome, whereas the precise ultrastructural localization of PV antigen remains unclear and controversial to date. To further investigate this question, we have compared the location of immune deposits in 10 patients with PF and 9 patients with PV by both direct and indirect immunoelectronmicroscopy (IEM). Inclusion criteria were based upon clinical features, histological level of cleavage and characterization of circulating antibodies by westernblot on epithelial bovine tongue extracts. IEM was performed on unfixed 0,7 mm thick slices of patient skin (Direct IEM) or normal human skin (Indirect IEM) and stained with peroxydase. In PF, by direct IEM (6/10) or by indirect IEM (10/10) immune deposits were located on desmosomes (desmoglea). In PV, by direct IEM (5/9) or by indirect IEM (8/9), deposits were situated on the extracellular part of the desmosome and along large portions of the keratinocyte membrane without desmosomal structures. These results suggest that, in contrast to PF, the target antigen in PV is not always restricted to desmosomes. As various types of adherens junctions have been described to mediate cell adhesion in the epidermis, the PV antigen could be a component of desmosomes and of other focal adhesions.

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A REPORT OF TWO CASES OF HALLOPEAU TYPE PEMPHIGUS VEGETANS WITH DETECTION OF ANTI-DESMOCOLLINS I AND II AUTOANTIBODIES BY IMMUNOBLOT ANALYSIS, Makoto Iwasa,[1] Koji Hashimoto,[1] Mari Higashiyama,[1] Takashi Hashimoto[2] and Kunihiko Yoshikawa,[1] [1]Department of Dermatology, Osaka University School of Medicine, Osaka, Japan and [2]Department of Dermatology, Keio University School of Medicine, Tokyo, Japan

Pemphigus foliaceus (PF) sera react with a 150 kD antigen, desmoglein (DG), while pemphigus vulgaris (PV) sera react with a 130 kD PV antigen. Recent molecular cloning studies have revealed that both DG and PV antigen are members of cadherin family of cell adhesion molecule and that PV antigen shows very high homology to DG. We have recently noticed that another desmosoal cadherin molecule desmocollins I and II (DCI/II), are also recognized by some pemphigus antibodies. In the present study we report two cases of Hallopeau type Pemphigus vegetans. Immunoblot study revealed that the sera of these cases reacted not only with the PV antigen but also with both DG (PF antigen) and DCI/II. Although the significance of these findings are not known at present, further studies for the antigen profile for more pemphigus vegetans patients may unravel the pathogenesis of this rare type of pemphigus.

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TRACE ELEMENTS IN HUMAN FINGERNAILS; NORMAL SUBJECTS VERSUS PEMPHIGUS PATIENTS. C. Solovan, V. Feier, N. Farbas, Department of Dermatology, University of Medicine and Pharmacology, Timisoara, Romania

Previous studies showed increasing interest in human fingernails with regard to the trace elements contained in this material and possible implications for medical purposes. Detection and quantitative determination of trace elements were performed by the electronic mycroprobe (emission of X-rays by electronic excitation) technique in 6 normal subjects (12 determinations) and 5 pemphigus vulgaris patients (20 determinations). About 9-11 elements (Si,S, Cl,K,Ca,Mn,Cu,Fe,Mg,Al,Zn) have been detected in human fingernails samples, taken from both hands and feet separately (parts per millio-ppm). The study showed a constant high content of S in the fingernails of normal subjects (≈40,000 ppm). The pemphigus patients showed a lower level of S in the fingernails which revealed a tendency to normalisation during treatment. Other constant trace elements faced were Cl,K,Ca with a wide range of values probably influenced by various factors (eg:nutritional,environment). Si,Mn,Cu,Fe were inconsistantly present while Mg,Al and Zn were totally absent with this method, as was Na.

In conclusion, we believed that the S content was implicated in the fingernail dystrophy of pemphigus patients.

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CHARACTERIZATION AND COMPARISON OF AUTOANTIBODIES AGAINST 230 AND 170 KD BULLOUS PEMPHIGOID ANTIGENS. Soo-Chan Kim, Bum Jun Lee, Dept. of Dermatology, Yonsei University Wonju College of medicine, Wonju, Korea

Bullous pemphigoid(BP) and herpes gestationis(HG) are immunologically closely related diseases and immunoblotting studies have shown that 30-50% of BP and 90% of HG sera recognize a common 170KD BP antigen. However, HG autoantibodies have characteristic features such as avid complement fixing capacity and IgG1 subclass predominance.

The aim of this study is to determine if there are any differences in complement fixing capacity and IgG subclass distribution between autoantibodies against 230 and 170 KD BP antigens, and to test the hypothesis that BP autoantibodies against the 170KD antigen have the same characteristics as the HG autoantibodies, what was previously called the "HG factor".

We determined the above characteristics by complement fixing IIF(indirect immunofluorescence) and plain IIF, respectively, in affinity purified antibodies specific to 230 and 170KD BP antigens prepared from a BP sera that has autoantibodies against both 230 and 170KD BP antigens. We also performed the same studies in sera characterized by immunoblot analysis from 25 BP and 2 HG patients.

Complement fixing capacity was present only in the affinity purified autoantibodies against 170 KD BP antigen but IgG4 was the dominant subclass in both affinity purified autoantibodies against 230 and 170KD antigens. This result agreed with our hypothesis only in respect to the complement fixing capacity. However, we found no differences in complement fixing capacity and the distribution of IgG subclasses between the sera against the 230 and 170 KD BP antigens characterized by the immunoblotting.

We conclude that there are no significant differences between the BP autoantibodies against the 230 and 170KD antigens and it is unlikely that BP autoantibdies against the 170KD antigen have the same characteristics as the HG autoantibodies.

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A NOVEL BULLOUS PEMPHIGOID ANTIGEN (BP125) IS LOCATED IN THE DEEPER LAYERS OF THE BASEMENT MEMBRANE ZONE. Shun-Qiang Gao and Jean-Claude Bystryn. The Ronald O. Perelman Department of Dermatology, NYU School of Medicine, New York, NY, USA and Fourth Hospital, Hei Bei Medical College, Shi Jia Zhuang, China.

This study, conducted to characterize BMZ antigens in different extracts of skin, unexpectedly revealed that some patients with bullous pemphigoid (BP) have antibodies to a novel 125 kD antigen in the deep layers of the BMZ.

Sera of 38 pts with BP and 55 control pts (17 BMZ antibody positive but without BP - 6 cicatrizing pemphigoid, 6 epidermolysis bullosa acquisita or bullous LE, and 5 combined staining sera, and 38 BMZ antibody negative pts) were tested by Western immunoblotting for antibodies in 4% SDS+2M urea extracts of the dermal and epidermal side of salt split normal human skin. Eight (21%) of the BP pts but none of the controls had antibodies that reacted strongly to a 125 kD antigen (BP125) in the extract of dermis. By indirect Immunofluorescence using affinity purified antibody, BP125 was localized to the BMZ on the dermal side of salt split skin. Based on the results of co-migration and specificity analysis, BP125 differs from the 230 kd and 180 kd BP antigens, the EBA antigen, the p84 dermal antigen defined by combined staining sera, and from epiligrin, laminin, type III and IV collagen, and keratins.

These results indicate that autoantibodies in BP can be directed to a novel 125 kD antigen located in the deep layers of the BMZ. The presence in BP of antibody responses to antigens located in different layers of the BMZ suggests that different immune mechanisms maybe involved in the pathogenesis of this disease.

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BULLOUS PEMPHIGOID IS STRONGLY ASSOCIATED WITH HLA-DQ7 IN MALES. SA George, V Venning, J Allen, [1]C Taylor, [1]K Welsh, F.Wojnarowska. Dept.of Dermatology and [1]Tissue Typing Laboratory, Churchill Hospital, Oxford

Immunogenetic studies in Bullous Pemphigoid (BP) have so far failed to show clear correlation with the HLA class I and class II gene products. Recent improvements in serological typing have prompted us to reevaluate its association with class II antigens. Typing was performed in 50 (22 M, 28 F) adult caucasians with BP confirmed by IgG binding to the epidermal side of salt split skin. Using standard NIH serological typing protocols, 19 men (86%) and 10 women (36%) were HLA-DQ7 positive. Control population is 30% and the frequency in males is therefore statistically highly significant ($p < .0001$, $pc < .001$). Validation of DQ7 typing was achieved in controls and patients using sequence specific amplification. No significant associations were detected with other HLA-DQ or DR antigens although DQ6 was reduced in males.

This is the first demonstration of a sex specific immunogenetic difference in a primary dermatological disease and so far we are unable to explain it. It is also the first demonstration of an immunogenetic association in BP. HLA-DQ7 positivity showed no correlation with coexisting autoimmune diseases, mucosal involvement or internal malignancy.

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CLINICAL CORRELATIONS AND PROGNOSIS BASED ON SERUM AUTOANTIBODIES IN CICATRICIAL PEMPHIGOID.
M. Nayarg F. Wojnarowska, Department of Dermatology, Churchill Hospital, Oxford, England.

31 patients with cicatricial pemphigoid (CP) were assessed for the presence of circulating basement membrane zone (BMZ) antibodies. Indirect immunofluorescence tests were performed using salt split human skin as antigen substrate. 14 patients (45%) were antibody positive while 17 (55%) were antibody negative. The possible association of selected clinical features such as chronicity of disease, severity, extent of mucosal involvement and scarring with the presence of antibodies were sought. There were no significant clinical differences between the two groups. Within the subset of antibody positive CP patients, 8(57%) had IgG, 4(29%) had IgG and IgA and 2(14%) had IgA alone. Other investigators have suggested that IgA may be a marker for severe mucosal disease and in the present study we tested this hypothesis. The IgA group of patients did not have increased mucosal involvement or scarring nor did they relate to any differeces in HLA type. There is considerable clinical diversity among CP patients but this does not correlate with specific antibody prevalence. The role played by BMZ antibodies in the pathogenesis of CP has not been clarified. Circulating BMZ antibodies when detectable assist in the diagnosis of CP but are of little value in predicting the natural course and outcome of the disease.

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HLA CLASS III ASSOCIATIONS IN PEMPHIGOID (HERPES) GESTATIONIS
M. M. Black, R. E. Jenkins, J. F. Shornick, B. S. Bhogal, C. M. Artlett * and K. I. Welsh *, St. John's Institute of Dermatology, St. Thomas' Hospital, London and *Tissue Typing Laboratory, Oxford, UK

Pemphigoid gestationis (PG) is a rare autoimmune bullous disease occurring during pregnancy or the puerperium. It is characterised by dense linear deposition of complement, with or without IgG, along the cutaneous basement membrane zone. It is associated with the MHC class II (HLA) antigens DR3 and DR4 (1). We have considered whether the HLA-DR association observed in PG actually reflects a more important association of "MHC class III" genes encoding complement components C2, factor B,C4A or C4B. Null alleles are frequent at both C4A amd C4B loci.

We have investigated by high voltage gel electrophoresis polymorphisms of factor B, C4A and C4B in 38 patients with PG confirmed using immunofluorescence.

Factor B allele was found in a similar proportion to controls but 34 (90%) cases carried a C4 null allele.

Our findings suggest that the C4 null allele may be the primary genetic association in PG and could explain the impaired handling of circulating immune complexes and autoantibodies that are a feature of the condition.

(1) Shornick JF, Stastny P, Gilliam JN. High frequency of histocompatibility antigens HLA-DR3 and DR4 in herpes gestationis. J. Clin. Invest. 1981; 68:553-555.

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OPTIMAL CONDITIONS OF 1 MOLAR SODIUM CHLORIDE SPLITTING TECHNIQUE TO DEMONSTRATE BASEMENT MEMBRANE ZONE ANTIGENS IN BULLOUS PEMPHIGOID, EPIDERMOLYSIS BULLOSA ACQUISITA AND LINEAR IgA BULLOUS DERMATOSES
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Separation of the dermo-epidermal junction through the lamina lucida is essential for immunofluorescent evaluation of subepidermal bullous diseases. Exposure to hypertonic NaCl is known to produce this split (1). However, some BMZ proteins are degraded during the procedure. This study was aimed to determine optimum splitting conditions for detection of anti-BMZ antibodies in bullous pemphigoid (BP), epidermolysis bullosa acquisita (EBA) and linear IgA bullous dermatoses (LABD). Normal skin from routine operations was incubated in 1M NaCl solution for: (a) 48 hours at room temperature; (b) 48 hours at room temperature with Phenyl methylsulfonyl fluoride (PMSF, a proteolytic enzyme inhibitor); (c) 72 hours at room temperature; (d) 72 hours at room temperature with PMSF; (e) 72 hours at 4°C with PMSF. Specimens were then employed as substrates for indirect immunofluorescence. Sixty-six patients (40 with BP, 6 with EBA and 20 with LABD) were studied.

All BP and those LABD (n=15) with epidermal-binding antibodies were best detected on skin incubated at 4°C with PMSF for 72 hours. These conditions did not improve the detection of all EBA and those LABD (n=5) with dermal-binding antibodies.

BP and epidermal-binding LABD antigens are therefore susceptible to degradation during incubation in 1M NaCl whereas those of EBA and dermal-binding LABD are not.

(1)Willsteed EM, Bhogal BS, Das A, Bekir SS, Wojnarowska F, Black MM, Mckee PH. An ultrastructural comparison of dermo-epidermal separation techniques. J. Cutan. Pathol. 1991;18:8-12.

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CLUSTERING OF EPIDERMOLYSIS BULLOSA SIMPLEX MUTATIONS IN RELATION TO DISEASE PHENOTYPE: DATA FROM WEBER-COCKAYNE EBS. F J D Smith[1], S M Morley[1], E L Rugg[1], H A Navsaria[2], I M Leigh[2], R A J Eady[3], M J Tidman[4], E B Lane[1]. [1]CRC Cell Structure Research Group, Dept Anatomy & Physiology, Univ of Dundee, UK; [2]Experimental Dermatology Labs, London Hospital Medical College, London, UK; [3]St John's Inst. Dermatology, UMDS, St Thomas' Hospital, London, UK; [4]Dermatology Dept, Royal Infirmary, Edinburgh, UK.

Epidermolysis bullosa simplex (EBS) of the severe Dowling-Meara (DM), Koebner or mild Weber-Cockayne (WC) types are all characterized by blistering within the epidermal basal keratinocytes in response to physical trauma. Point mutations in the basal keratins K5 and K14 have been shown to be responsible for DM-EBS. We have now identified the mutations in keratin genes in 9 families with EBS, including several classical WC-EBS, with supporting data collected from clinical examinations, electron microscopy and genetic linkage analysis. In contrast to the more severe DM-EBS we have observed that the milder WC form is associated with mutations in sites other than the extreme ends of the rod domain. Taken together with data from BCIE studies (see McLean et al, this meeting), 6 mutational clusters can now be identified. 2 clusters are clearly DM-associated, but our results suggest that a further 4 clusters (including 2 novel ones) are characteristic of milder disease phenotypes. This includes a second site in helix 1A, C-terminal to the hotspot known to be associated with DM-EBS, where a new K5 Asn-Lys193 mutation consolidates a recently-reported K14 mild phenotype mutation (Nature Genetics 3: 327, 1993) to establish this as a distinct site. One interpretation of the mutation clusters is that they indicate important sites at which sequence variation is especially detrimental to keratin filament stability or strength.

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PCR-BASED DETECTION OF INTRAGENIC RFLPs IN THE HUMAN TYPE VII COLLAGEN GENE: APPLICATIONS TO PRENATAL DIAGNOSIS OF DYSTROPHIC EPIDERMOLYSIS BULLOSA. Yoshiko Tamai, Xin Zhang, Angela M. Christiano, *Alain Hovnanian and Jouni Uitto. Department of Dermatology, Jefferson Medical College, Philadelphia, PA 19107; and *Hopital Henri Mondor et INSERM, Creteil, France.

We have recently completed characterization of the complete cDNA and corresponding gene of human type VII collagen. Our search for mutations in patients with dystrophic EB has led to the observation of several polymorphic sites in the exons of the COL7A1 gene, as well as several mutations. Two of these sites (PvuII and AluI) were previously reported (Christiano et al., Genomics 19:827-828, 1992) and four additional RFLPs can be recognized by the enzymes StyI, MspI and MnlI. Each of these RFLPs can be identified by PCR amplification of genomic DNA, followed by restriction enzyme digestion and agarose gel electrophoresis. We recently performed an early prenatal diagnosis of RDEB based on linkage analysis in one family. The PvuII RFLP was informative in two parents and an affected child with RDEB. A transabdominal chorionic villus biopsy was performed at 10 weeks gestation and analysis of the PvuII RFLP revealed that the fetus is a normal carrier. The pregnancy will be carried to term and delivered in 9/93. PCR-based detection of several RFLPs in the COL7A1 gene is a rapid first-trimester alternative to fetoscopic skin biopsy, which was previously the only option available to families with an RDEB child. These techniques provide a major advance in the prenatal diagnosis of RDEB, and will also be useful in preimplantation diagnosis of RDEB in the future.

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IN SITU DEGRADATION OF BASEMENT MEMBRANE BY RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA FIBROBLASTS AND FIBROBLAST-DERIVED METALLOPROTEINASES. Rudd, Rebecca L., Krejci, Niels C., Bruckner-Tuderman, Leena,* Bauer, Eugene A., McGuire, Joseph
Department of Dermatology, Stanford University School of Medicine and University Hospital Zürich*

The ability of recessive dystrophic epidermolysis bullosa (RDEB) fibroblasts to degrade basement membrane zone components on dermis in situ was examined. Fibroblasts from three RDEB patients and one normal control were placed on acellular dermis retaining basement membrane zone (BMZ). After two weeks, RDEB fibroblasts had migrated into the dermis. The BMZ showed diminished or loss of staining for type VII collagen, type IV collagen and laminin in the region containing fibroblasts. Normal fibroblasts showed little migration into dermis. Dermis with normal cells showed continuous linear staining of these BMZ components, and the staining was comparable to that seen in dermis without cells. In order to identify the enzymes responsible for this effect, degradation of BMZ by purified matrix metalloproteinases (MMPs) in situ was examined in sections of normal human foreskin. Stromelysin and interstitial collagenase individually or in combination reduced or abolished interaction of BMZ with an antibody to the helical domain of type VII collagen. However, staining of the carboxyl terminus of type VII collagen was not diminished. Staining for linkin, a microthread-like filament proposed to be attached to type VII collagen, was also abolished. Staining for type IV collagen was not reduced by these MMPs. The effect of stromelysin was shown to be independent of indigenous collagenase in the skin. These results point to the importance of MMPs in the pathogenesis of RDEB.

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EXCLUSION OF LINKAGE OF HAILEY-HAILEY DISEASE TO THE TYPE I AND II KERATIN GENE CLUSTERS. Elizabeth A. Welsh, Jeanette M. Bonifas,** John W. Bare,** David T. Woodley,* and Ervin H. Epstein, Jr.,**
Department of Dermatology, Stanford University School of Medicine, CA, *Northwestern University Medical School, Chicago, IL, and **University of California, San Francisco, USA.

Hailey-Hailey (Familial Benign Chronic Pemphigus) disease is a rare disorder inherited in an autosomal dominant fashion and is characterized by blisters caused by suprabasal epidermal acantholysis. Because of the reported changes in suprabasilar keratin gene expression in Hailey-Hailey disease, we have used linkage analysis to test whether keratin gene mutations might underlie Hailey-Hailey disease in one large multi-generation family. All available family members were genotyped at polymorphic microsatellite repeat loci mapped to the regions of the type I and II keratin gene clusters on chromosome 17q and 12q, respectively. Genotype analysis excluded linkage of the inheritance of Hailey-Hailey disease to the region of chromosome 12q containing the genes encoding the type II keratins (LOD < -2.0 at a recombination fraction of 0.20.) In addition, analysis of polymorphic loci in the region of the type I keratin gene cluster on chromosome 17q provided evidence against linkage in the family studied. These results suggest that keratin gene mutations do not underlie the pathogenic phenotype of Hailey-Hailey disease. Studies are in progress to analyze other candidate genes in this manner in an attempt to identify the gene(s) whose abnormalities may contribute to the pathogenesis of Hailey-Hailey disease.

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EPIDEMIOLOGICAL OBSERVATIONS OF ACQUIRED BULLOSUS DERMATOSES IN MACEDONIA(1977-1992). Aleksandar Ančevski, Marija V`čkova-Laskoska, Lozena Zaharieva. Clinic of Dermatology and Venerealogy, Medical Faculty, Skopje, Republic of Macedonia.

The article deals with acquired bullosus dermatoses in Macedonia in the course of the last 15 years. Special attention is paid to Pemhigus vulgaris, Pemhigoid bullosus, Dermatitis herpetiformis Duhring and Necrolysis epidermalis toxica Lyell. It isshown a demographic map of Macedonia. The results obtainedare represented in tables and graphically. All cases have been hystologically verified and the majority of them have been immunologically verified. The incidence of pemhigus vulgaris in Macedonia is 0.37 newly discovered cases of 100.000 inhabitants per year, pemhigoid bullosus-0.15, dermatitis herpetiformis-0.08. There has not been discovered a significant difference in the incidence between the Macedonian population and the Albanian and other nationalities. In the entire investigated material with pemhigus vulgaris the most frequent age group of patients has been between 41 and 50 years of age, with pemhigoid bullosus over 70 years of age. In patients with dermatitis herpetiformis Duhring there has not been found any significant difference for certain age groups.

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ETHNIC DIFFERENCE OF VARIED STRATUM CORNEUM FUNCTION IN RELATION TO STRATUM CORNEUM LIPIDS. Kiyoko Sugino, Genji Imokawa and Howard I. Maibach. Department of Dermatology, University of California, San Francisco, CA and Kao Biological Science Laboratories, Tochigi, Japan

Ceramides, a major component of stratum corneum(SC) lipids, play an important role in both water-holding(WH) and permeability barrier functions(BF) of the SC. To assess the difference in physiological responses between races with special reference to ceramides in the SC, the susceptibility of the SC to irritants was studied by 24 hr occlusive patch testing in 4 races, Caucasian, African American, Hispanic American and Asian, for WH and BF. Capacitance measurement at 72 hr following patch testing using 5% HCl and 2% SLS revealed that Caucasian and Hispanic American have a reduced tolerance toward the disruption of WH properties compared with African American and Asian. Similarly, TEWL measurement at 72 hr following the same treatment demonstrated that Caucasian is most susceptible to damages in the skin BF, followed by Hispano American, African American and Asian. As a basis for explaining the functional difference, we quantified the casual level of SC lipids including ceramides in 4 races, accompanied by the normal level of the SC functions. SC lipids, especially ceramides, assayed as μg/mg SC, are significantly lower ($p<0.05$) in African American compared with other races. Measurement of TEWL exhibited the decreasing order in African American > Caucasian > Hispanic American > Asian. Water content showed higher Asian values, lower Caucasian, African American and Hispanic American values, showing a consistency with the lower ceramide level in African American. These findings, taken together, suggest that the constitutive functional differences of the intact SC among different races are associated with ceramide dynamics, but their vulnerability is not explained only in terms of the content of the SC lipids.

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FOAM CELL INDUCIBLE LIPOPROTEINS IN EXPERIMENTAL XANTHOMA TISSUES. Mitsunori Ikeda, Kozo Okawa, Yasuhiko Hirata and Hajime Kodama, Department of Dermatology, Kochi Medical School, Okohcho, Nankoku, Kochi 783, Japan

To clarify the mechanism of the recruitment of foam cells in xanthoma lesions, we examined the lesional lipoproteins taken up by macrophages. The LDL fraction was extracted from rabbit experimental xanthoma tissues by ultracentrifugation. The LDL fraction was shown to be incorporated into mouse peritoneal macrophages, as cholesterol esterification was stimulated. Macrophages were transformed to foam cells by incubation with the xanthoma-derived LDL for 72 hours. Human serum LDL oxidatively modified by incubation with the experimental xanthoma tissues was also taken up by mouse peritoneal macrophages. Both the xanthoma-derived LDL and the modified human LDL were more negatively charged and contained much higher levels of lipid peroxides than native LDL. These findings indicate that extravasated lipoproteins are oxidatively modified in xanthoma lesions and that the modified lipoproteins contribute to the conversion of infiltrating macrophages to foam cells within xanthoma lesions.

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PROCESSES OF LOW DENSITY LIPOPROTEIN OXIDIZATION IN EXPERIMENTAL XANTHOMA TISSUES. Kozo Okawa, Mitsunori Ikeda, and Hajime Kodam Department of Dermatology, Kochi Medical School, Kochi, Japan

In vitro studies have shown that oxidatively modified LDL plays important roles in foam cell infiltration. Reactive oxgen species, phospholipase A2 and oxidases (lipoxygenase and cyclooxgenase) are the candidates that mediate the process of LDL oxidization in xanthoma lesion. In the previous study, human LDL was revealed to become more negatively charged and contain a larger amount of lipid peroxides than native LDL by incubation with experimental xanthoma tissues. Mouse peritoneal macrophages were shown to incorporate the oxidatively modified LDL. In the current study, several kinds of antioxidants (superoxide dismutase, butylated hydroxytoluene, catalase and α-tocopherol) and inhibitors for phospholipase A2 and oxidases were investigated on their effects on the LDL oxidization. Every antioxidants and oxidase inhibitors inhibited the LDL oxidization dose-dependently. In addition, phospholipase A2 inhibitor also showed the inhibitory activity. These findings indicate that extravasated LDL receives oxidative modification in the xanthoma tissues by both reactive oxgen species and oxidases. Phospholipase A2 might play a role in the LDL oxidization.

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AGING AND THE SEASONS INFLUENCE STRATUM CORNEUM LIPID LEVELS. *A. Rawlings, A. Mayo, J. Rogers, *I. Scott, *Unilever Research, Edgewater, NJ, USA; Unilever Research, Colworth House, Sharnbrook, Bedford, UK

Stratum corneum lipids play a predominant role in stratum corneum barrier, mechanical together with desquamatory properties. However, measurements of changes in stratum corneum lipid levels with age on different body sites are contradictory and no studies have been performed examining the variation in stratum corneum lipid levels due to the seasons. It is possible that aging and seasonal variations in epidermal lipid biosynthesis could lead to changes in stratum corneum lipid levels and may contribute to the worsening of skin condition and xerosis.

To understand the effects of these variables for their possible impact on skin condition, we chromatographically examined stratum corneum lipids of healthy Caucasian facial and hand skin with increasing age (20-50 years) together with lipid levels of healthy Caucasian hand stratum corneum in the winter, spring and summer months of the year. A dramatic variation in stratum corneum lipid levels was apparent from the studies. In the aging study, a decrease in the mass levels of ceramides, cholesterol and fatty acids was seen in the younger age group compared with the older age group (approximately 65% and 40% for each lipid class on the face and hands respectively). In the seasonal study ceramides, cholesterol and fatty acid levels were decreased by approximately 50% and 25% in the winter and spring months of the year compared with the summer season.

Aberrations in stratum corneum lipid levels and membrane structure have been identified in winter xerosis. It is possible that the reduced levels of lipids in the stratum corneum as a result of aging and seasonal variations may be a predisposing factor in xerosis. The lower levels of stratum corneum lipids will probably be more easily extracted, or their structure more easily perturbed, leading to abnormalities of stratum corneum function.

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FUNCTIONAL ANALYSIS OF CERAMIDASE AND SPHINGOMYELINASE IN THE STRATUM CORNEUM OF PATIENTS WITH ATOPIC DERMATITIS. Yasuko Ogawa, Kumi Jin, Yuko Higaki, Makoto Kawashima, Kazuhiko Higuchi, Yutaka Takagi, Yukihiro Yada and Genji Imokawa, Department of Dermatology, Tokyo Women's Medical College, Tokyo and Kao Biological Science Laboratories, Tochigi, Japan

To elucidate the mechanisms which are involved in the decrease of ceramides in the stratum corneum (SC) of patients with atopic dermatitis (Imokawa et al, J.I.D. 96: 523, 1991), we examined the both activities of sphingomyelinase (SMase), which is a major enzyme in ceramide production, and of ceramidase (CDase) which is an essential enzyme in ceramide degradation, in the SC. The specimens of the SC of forearm skin were obtained by tape-stripping from healthy volunteers (n=61, 0 to 84 y-o) and atopic uninvolved and involved skin (n=23, 0 to 27 y-o). SMase activity in the SC extract was estimated using [N-methyl-^{14}C] sphingomyelin as the substrate. CDase activity was determined using ^{14}C-palmitoylsphingosine. Among the atopic uninvolved and involved SC samples, CDase activities showed no significant differences over age-matched controls. In contrast, the activities of SMase were 7.1 and 32.4 times higher in uninvolved and involved atopic skins, respectively, than those of age-matched healthy controls. TLC analysis of enzymically decomposed materials revealed that whereas the activities in age-matched control were attributed to SMase which releases phosphorylcholine (PC), atopic SC samples contain undetermined enzyme activities of releasing chemicals other than PC from sphingomyelin, which may lead to the seemingly elevated activities of SMase. These findings suggest that sphingomyelin metabolism is definitely altered in atopic dermatitis, resulting in the decrease of ceramides in the SC.

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THE INVESTIGATION OF PHOSPHOLIPIDS AND FATTY ACID OF RED BLOOD CELL(RBC) MEMBRANE AND SERUM IN PATIENTS WITH ATOPIC DERMATITIS(AD) Akira Fujioka [1], Yuko Hamada[1], Hiroshi Takasu[1], Kensei Katsuoka [1], Shigeo Nishiyama [1], Tadaaki Nagoya[2] and Shojiro Tsukamoto[2], [1]Department of Dermatology, Kitasato University School of Medicine, Sagamihara, [2] Department of Common-Use Facilities for Medical Research, Nihon University School of Medicine, Tokyo, Japan

It has been reported that Linolenic Acid(LA) can not be converted to Gamma-Linolenic Acid(GLA) by impaired supply of delta-6-desaturase in AD. The purpose of this study is to estimate whether or not the inflammatory findings in AD have relation to lipid metabolism of RBC membrane or serum. Phospholipids of RBC membrane and serum from severe AD and normal control were divided into 6 components(phosphatidylethanolamine(PE), phosphatidylinositol(PI), phosphatidylserine(PS), phosphatidylcholine(PC), sphingomyelin(SM), lysophosphatidylcholine(LPC)) using thin-layer chromatography separation. The chromatogram was developed with chloroform/methanol/acetic acid/water(50:30:4:2). Fatty acid composition of each components of phospholipids were examined by gas chromatography. The quantities of PE and PS could be recognized in inside of cell membrane were large in AD. Analysis in PC and SM located in outside of cell membrane was just the opposite to it. Short-chain saturated fatty acids were decreased in RBC membrane of AD, but they were increased in serum. Some of polyunsaturated fatty acids were incresed in RBC membrane of AD. However, no significant difference was found in the ratio of LA and GLA between AD and control. These data suggest that fluidity of cell membrane or cell activation is changed in AD, whereas the role of LA and GLA in AD is not exactly known.

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SKIN SURFACE LIPID PEROXIDATION IN ATOPIC DERMATITIS. Yoshiyuki Kohno, Motoji Takahashi, Junko Ohsawa*, Kazuko Kitamura* and Zenro Ikezawa*, Shiseido Research Center, Yokohama, Japan, *Department of Dermatology, Yokohama City University School of Medicine, Yokohama, Japan.

Recently, lipid peroxidation has drawn much attention of the dermatologist because of its importance in pathological and etiological studies on skin diseases and aging. We have studied skin surface lipid peroxidataion in atopic dermatitis (AD) patients (n=60) and normal subjects (n=45). Lipid was collected from forehead, neck and back sites by gently wiping skin surface by cotton impregnated with acetone. Lipid peroxides were investigated using a CL-HPLC (high performance liquid chromatography with chemiluminescence detection system). We have found that squalene is the first target lipid on skin surface by an oxidative stress and that a conversion ratio of squalene to squalene mono-hydroperoxide is good parameter to indicate the degree of peroxidation of skin surface lipids. In AD patients, the conversion ratio was significantly higher than that in normal subjects taken as control. And in several cases of AD, other lipids than squalene were also heavily peroxidized. Furthermore, clinical scores for dryness and scaling in AD patients became severer along with the increase of the conversion ratio. From these results it was concluded that the antioxidative potential of AD skin is inferior to that of normal skin and it is presumed that the antioxidative potential is related to the skin disorders such as dryness and scaling.

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EFFECTS OF HIGH DOSE ORAL THERAPY WITH EVENING PRIMROSE OIL IN SEVERE ATOPIC DERMATITIS. Johannes Hollmann, Silke Michelsen, Thomas Jansen, Gerd Plewig and Frank Rippke*, Department of Dermatology, Ludwig-Maximilians-University, Munich, Germany, and Beiersdorf Co.*, Hamburg, Germany

In an open uncontrolled study, 10 patients (8 men and 2 women) at the age of 18 - 60 years with severe atopic dermatitis were treated for 12 weeks with daily 5.6 - 6.4 g evening primrose oil (Epogam 2 x 6 capsules), which is equivalent to 480 mg gamma-linolenic acid. Every 4 weeks clinical and serological parameters, transepidermal water loss (TEWL), surface lipids and stratum corneum lipids were examined. TEWL was measured by electrolytic water analysis with the SPR-DMU (Meeco). Plantar stratum corneum was delipided by a modified method of Folch et al. Skin surface lipids from the elbow were extracted with aceton. The main lipid fractions including phospholipids, cholesterol sulfate, glucosylceramides, six major ceramide fractions, free sterols, free fatty acids, triglycerides, sterol esters, and n-alkanes were separated and quantified after stepwise development of a single silica gel HPTLC- or TLC-plate using three consecutive solvent systems. Hitherto not performed quantitative and qualitative changes of intercellular stratum corneum lipids were calculated. The percent extension of the eczema was significantly reduced from 56.1% to 21.7% ($p < 0.001$). The general eczematous state compared to the state before therapy (scored with Abrams' scale) showed an increasing improvement ($p = 0.003$). Serological parameters including IgE-levels and RAST-classification of the main immediate-type allergens demonstrated no noticeable changes. A significant reduction of TEWL was found both at the forearm and subscapular area ($p < 0.001$).

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MONOHYDROXY FATTY ACIDS IN SPECIFIC EPIDERMAL PHOSPHOLIPIDS: DEPLETION IN LESIONAL PSORIATIC SKIN. Grøn B, Iversen L, Ziboh V, Kragballe K. Department of Dermatology, University of Aarhus, Aarhus, Denmark.

The monohydroxy derivatives of linoleic acid and arachidonic acid play a role in the regulation of epidermal proliferation and skin inflammation. Although these monohydroxy fatty acids (MHFAs) are rapidly incorporated into specific phospholipids, increased levels of free MHFAs have been detected in psoriatic skin. The purpose of the present study was to determine the pools of unesterified and esterified MHFAs in keratomed epidermal slices, taken from normal skin as well as lesional and non-lesional psoriatic skin. Extracted phospholipids were separated by thin layer chromatography. The isolated fractions of phosphatidylcholine (PC), phosphatidylinositol (PI) and phosphatidylethanolamine (PE) were then treated with phospholipase A_2 to release fatty acids in the sn-2 position. The released monohydroxy fatty acids were separated by reversed phase and straight phase high performance liquid chromatography. They were identified as the monohydroxy derivaties of linoleic acid: 9-hydroxy-octadecadienoic acid (9-HODE) and 13-hydroxy-octadecadienoic acid (13-HODE) and as the monohydroxy derivative of arachidonic acid : 15-hydroxy-eicosatetraenoic acid (15-HETE). These findings were consistent with the presence of unesterified 9-HODE, 13-HODE and 15-HETE. In contrast, 12-hydroxy-eicosatetraenoic acid (12-HETE), although found to be present in high amounts as unesterified 12-HETE, was not detectable in the sn-2 position of the specific phospholipids analyzed. When comparing matched pairs of non-lesional and lesional psoriasis skin, the levels of 9-HODE, 13-HODE and 15-HETE esterified to the sn-2 position of PC, PI and PE were significantly lower (2-3 fold) in the lesional psoriatic skin samples. This depletion of MHFAs in specific phospholipids of lesional psoriasis skin may be due to an imbalance between phospholipase and acyltransferase activities. Because the levels of esterified MHFAs may influence signal transduction and eicosanoid metabolism the described changes may be relevant for the inflammatory processes occuring in psoriasis.

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THERE IS A CELL-BOUND LIPASE WITH ACIDIC OPTIMUM PH VALUE IN PROPIONIBACTERIUM ACNES. Satoshi Dekio and Joji Jidoi, Department of Dermatology, Shimane Medical University, Izumo, Japan

According to the previous studies, there are two kinds of lipases in Propionibacterium acnes (P. acnes); free lipase, the lipase activity of which is free from P. acnes cells, and cell-bound lipase, which shows the activity on the cells. The peak of free lipase activity was located only in the basic pH area. However, it has not been known yet where the optimum pH value of the cell-bound lipase is located. We thus studied on the optimum pH of the cell-bound lipase for more understanding of P. acnes skin infections. Two P. acnes strains, ATCC 11827 and ATCC 11828, were cultured in peptone-yeast extract-glucose medium with Tween 80. Lipase activities of the harvested cells were examined using the modified Whitaker's method under various pH conditions. The cells of both the two P. acnes strains showed two optimum pH values of lipase activity, pH 6.0 and pH 8.0. This means that there are two kinds of cell-bound lipases in P. acnes; one with an optimum pH at the acidic area, and another with that in the basic area. Considering that under proliferation of P. acnes, its circumstance has become acidic, the cell-bound lipase with an optimum pH value at 6.0 may play important roles in the course of progression of P. acnes skin infections.

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WATER-HOLDING CAPACITY AND LIPID COMPOSITION OF STRATUM CORNEUM IN ACNE VULGARIS. Kaoruko Takenouchi, Ayako Yamamoto, Kyoko Togashi and Masaaki Ito, Department of Dermatology, Niigata University School of Medicine, Niigata, Japan

The pathogenesis of acne vulgaris is not fully understood. We have investigated the possible comedogenic mechanism of water-holding dysfunction, since decreased water-holding capacity(WHC) of stratum corneum(SC) cause abnormal keratinization. Twenty-seven male patients, aged 14 to 20 years, who had mild or moderate acne and 40 control subjects were studied to examine the interrelationship between acne, WHC and lipid composition of SC. Using a hydrometer, electrical conductance value of SC was measured on the cheeks. SC lipids were extracted from the cheeks by a cup method and determined by high-performance thin-layer chromatography(HPTLC)/photodensitometry. Free sphingosine was also isolated and quantitated by TLC using ninhydrin to detect free amine group. The WHC was significantly lower in acne patients than in control subjects. Comparing the severity of acne, the WHC was lower in the patients with moderate acne. The total amount of accumulated lipid from the patient with moderate acne showed higher levels than those from control subjects. However, lowest lipid levels were obtained for the patients with mild acne. Furthermore, decreased levels of sphingolipids, which are believed to be important for WHC, were observed in acne patients. These results suggest that one of the primary events in comedogenesis may be abnormal keratinization of epithelium caused by diminished WHC.

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DISTRIBUTION OF 4-HYDROXY-2-NONENAL-MODIFIED PROTEIN IN EXPERIMENTAL BURN AND HUMAN SKIN TUMOR. Yukari Nakakuki[1)2)], Chikako Nishigori[1)], Shinya Toyokuni[2)], Koji Uchida[3)], Hiroshi Hiai[2)], and Sadao Imamura[1)]. Department of Dermatology[1)] and Pathology[2)], Kyoto University Faculty of Medicine, Kyoto, Japan, Department of Applied Biological Science[3)], Nagoya University School of Agriculture, Nagoya, Japan.

4-Hydroxy-2-nonenal (HNE) is one of the major products of membrane peroxidation. A number of adverse biological effects against protein and DNA including genotoxicity and mutagenicity in several systems have been shown. Recently, one of the authors (K.U.) has demonstrated that histidine (His) residues of proteins are important targets for the modification by HNE, and prepared a polyclonal antibody against HNE-His adduct. By the use of this antibody, we studied the distribution of HNE-modified protein in experimental burn model using 6wk-old male Wistar rats (130-150 g) and in several kinds of human skin tumors such as squamous cell carcinoma , malignant melanoma and Paget's disease. One and 3 hrs after burn, positive reactions were demonstrated in the cytoplasm of follicular epithelial cells and infiltrating inflammatory cells, suggesting that HNE-modified protein worked for cytotoxicity since degeneration of follicular epitheliumbecame remarkable not immediately, but 3 hrs after burn. Among human skin tumors studied, positive reactions were seen in Paget cells, but not in squamous cell carcinoma and melanoma cells. Paget cells, especially large Paget cells, showed cytoplasmic positive reaction in 10 of 14 specimens.

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ICAM-1 MOIETY IS CONSTITUTIVELY EXPRESSED AT LOW LEVELS BY RESIDENT UNSTIMULATED KERATINOCYTES "IN VIVO". AN "IN SITU" IMMUNOELECTRONMICROSCOPY QUANTITATIVE STUDY ON RESTING NORMAL HUMAN EPIDERMIS. Antonietta Lonati(1), Mieke A. Mommaas (2), Giorgio Pasolini (1), Antonio Lavazza (3), Mauro Marcelli (1), Bert J. Vermeer (2), Giuseppe De Panfilis (1); (1)Department of Dermatology, University Hospital, Brescia, Italy; (2)Department of Dermatology, University Hospital, Leiden, The Netherlands; (3) Istituto Zooprofilattico, Brescia, Italy

In previous investigations we demonstrated by immuno-electron-microscopy (IEM) performed on suspended epidermal cells (EC) that ICAM-1 is expressed on the cell surface of a small subpopulation of keratinocytes (KC) freshly isolated from normal human epidermis. However, such ICAM-1 expression on isolated KC might be non constitutive, but due to ICAM-1 "de novo" synthesis by KC stimulated by cytokines released by trypsinized EC, or conversely, the ICAM-1 molecule might even be sensitive to trypsin degradation. The aim of the present study was therefore to investigate the constitutive ICAM-1 expression by resting unstimulated KC in normal skin "in situ", i.e. without trypsinization and isolation of EC. For such a purpose, we utilized the most suitable and sensitive IEM system for the "in situ" immunolocalization of antigenic moieties, namely the immuno-colloidal-gold labeling of tissue ultracryosections. ICAM-1 positive KC resident within resting normal skin were detected by the presence of gold particles within the cytoplasm and especially along the plasma membrane. The numbers of gold granules were scored for a semi-quantitative analysis of the antigen expression. The semi-quantitative analysis of 100 resident KC visualized within ultracryosections of normal human skin and scrutinized, cell by cell, under the electron microscope, revealed that a number of 76.98 ± 88.38 gold particles was detectable within the cytoplasm and this score was highly significant as compared to controls ($p<0.0001$). Even more interesting a percentage of 15% KC showed the presence of gold particles also along the plasmacell membrane although a density per KC section (mid plane) of merely 16.46 ± 12.30 particles was scored (controls: no particles in any KC membrane), indicating that the amounts of ICAM-1 moieties expressed on the surface of this KC subset are presumably low. Thus this "in situ" IEM investigation clearly shows that ICAM-1 moieties are constitutively expressed, although at low levels, by a subset of resident KC "in vivo", even in resting unstimulated conditions. Such a low constitutive ICAM-1 expression by resident KC is, on the other hand, non surprising keeping in mind that a number of epithelial tissues contitutively express ICAM-1, the universal feature on all these cells being ICAM-1 induction from very low ICAM-1 levels on unstimulated cells to very high ICAM-1 levels triggered by mediators released at sites of inflammation. The currently demonstrated ICAM-1 expression on a subset of KC in normal skin can well account for the trafficking to and from normal epidermis of cell expressing ICAM-1 ligands, such as migrating Langerhans cells and occasional leukocytes. On the other hand the limited ICAM-1 expression only on a small KC subpopulation in normal skin can account for prevention of inappropriated KC/leukocytes interactions in the resting cutaneous immune environment, to prevent exaggerated cell-cell interactions which might alter the normal balance of controls whithin the cutaneous immune system.

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A CONCERN OF EPIDERMAL LANGERHANS CELL AND LATEX BEADS. Mamoru Masutani, Department of Dermatology, Fujita Health University School of Medicine, Aichi, Japan

When horseradish peroxidase(HRP) and ferritin(F) were inoculated as well as tuberculin test into the back of skin with guinea pigs, epidermal Langerhans cell(LC) could be taken up those materials though Langerhans cell granule(LCG) which was made by own cell membrane. Therefore, it is consideration that LCG comes finally to be secondary lysosome (phagolysosome). We have been observed the various epidermal cell activity with great and small sizes of latex beads(LB) (0.109 and 0.794um) which has no protein within only foreign body. This time, it will be described the evidence. A great part of LB was uptake into the dermal phagocyte(PC) on the observation. And then, PC went up to the epidermis throughout a basal lamina. LB different from HRP and F can not directly take by LCG in LC. However, foreign body within phagocyte in the epidermal region was a target for the LC. It is considered as one of the immunological ability of LC. In the above consequence, epidermal LC was meant antigen presenting cell.

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HYDROXYETHYLSTARCH DEPOSITS IN SKIN AND OTHER ORGANS. Jurecka W.[1], Szepfalusi Z.[2], Sirtl C.[3], Laubenthal H.[3], Scheiner O.[2], Kraft D.[2]; [1]Department of Dermatology and [2]Department of General and Experimental Pathology, University of Vienna, Austria, and [3]Clinic of Anaesthesiology, University of Bochum, Germany

Hydroxyethylstarch (HES) deposits have been described in various organs after its administration in haemodilution therapy. Itching is a well documented side effect and is more common after administration of higher doses. Up to now storage of HES has been demonstrated by specific immunohistochemical methods only in itchy human skin.

From 7 patients suffering from severe pruritus after treatment with HES for otological or neurological disorders skin biopsies were obtained. From further 15 patients undergoing repeated surgery due to several malignancies biopsies were obtained from skin, intestine, striated muscle, spleen, and liver at several time intervals after administration of HES (up to 53 months). From these patients only one showed pruritus. Specimens were fixed and embedded routinely for light microscopy and electron microscopy. A polyclonal rabbit antiserum against HES was used for light microscopical immunohistochemistry and immunoelectronmicroscopy to demonstrate HES storage in tissue. Storage of HES could be demonstrated in the skin of all patients, however, with less intensity after longer intervals. In intestine, striated muscle, spleen and liver storage was demonstrable only for about 2 years and was negative after longer intervals.

These results show, that storage of HES also occurs in non-itchy skin and that development of pruritus must also be dependent from individual factors of the patients. As pruritus disappears after several months and HES deposits are reduced slowly during this time in the skin, also quantitative factors of HES storage may be important for development of pruritus. These considerations may be important while studying HES induced pruritus.

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THREE-DIMENSIONAL MICROVASCULAR ARCHITECTURE OF SINUS HAIR: A SCANNING ELECTRON AND CONFOCAL LASER SCANNING MICROSCOPIC STUDY. Shigeaki Sakita, *Osamu Ohtani, and Masaaki Morohashi, Department of Dermatology and *Anatomy, Faculty of Medicine, Toyama Medical and Pharmaceutical University, Sugitani, Toyama, Japan

We have demonstrated the three-dimensional microvascular architecture of the sinus hair of the adult Wistar rat using scanning electron microscopy of vascular corrosion casts and confocal laser scanning microscopy (CLSM). Vascular corrosion casts were prepared according to the method introduced by Murakami. CLSM was performed on thick frozen-sections which were incubated with anti- VIII factor related antigen antibodies and then with FITC-labelled goat anti-rabbit IgG. CLSM was also conducted on the sections stained with TRITC-labelled phalloidin. The hair was surrounded by the sinus which consisted of the superficial ring sinus (RS) and the deeper cavernous sinus (CS). Many trabeculae existed in the CS but not in the RS. The hairshaft within the CS is surrounded by a basket-like capillary network. This network was densest at its lower part. The capillary network surrounding the lower part of the hairshaft was supplied by the radical artery and drained into the radical vein, while that surrounding the upper part of hairshaft was supplied by the subcapsular artery and drained into the subcapsular vein. The radical artery and vein passed through the bottom of the CS, while the subcapsular artery and vein penetrated the lateral wall of the CS. At the inside of the RS, only a few capillaries ascended along the hair and gave rise to the capillaries located below the epidermis. Our results indicate that the lower part of the sinus hair, which is the most important area for hair growth, is supplied with more blood and protected from external forces by the CS with many trabeculae. The present study has also shown that CLSM is a powerful tool for investigations on three-dimensional microvascular architecture.

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ABNORMALITIES IN THE DERMAL PAPILLA FROM CLINICALLY NORMAL HAIR FOLLICLES OF ALOPECIA AREATA PATIENTS MAY INDICATE AN AETIOLOGICAL ROLE IN THE DISEASE. [1]M. Nutbrown, [2]S. Macdonald Hull, [2]W.J. Cunliffe and [1]V.A. Randall, Departments of [1]Biomedical Sciences, University of Bradford, Bradford. [2]Dermatology, The General Infirmary, Leeds, UK

The aetiology of alopecia areata is not understood, but a subclinical condition has been described (Macdonald Hull et al. J Invest. Dermatol. 96:637-681, 1991) in which features seen in follicles from active lesions of alopecia areata scalps were also found in follicles from clinically normal, that is non-balding, regions. Ultrastructural investigations of such follicles should detect early cell changes and may indicate the primary sites of injury. Therefore, the ultrastructure of the dermal papilla has been examined in follicles from clinically normal regions and active edges of alopecia areata scalps of six patients and six controls

The well organised dermal papilla cells of control follicles were of the fibroblast type; the nuclei were usually large and ovoid and the cells had well developed rough endoplasmic reticulum and Golgi apparatus. The papilla basal lamina was of a regular pear shape and well defined three-layered structure. Although many dermal papillae of non-balding follicles retained properties similar to normal, there was a wider variation in papilla shape, basal lamina thickness and appearance of the cells. Some dermal papillae showed changes in both cells and extracellular matrix. Dermal papilla cells from follicles of active lesions had in most cases lost their fibroblast shape; they had very irregular profiles and very evenly stained, but distorted nuclei. Many cells contained pigmentary granules and circular lucent spaces and had weak and degenerate organelles. Basal laminae tended to be misshapen, diffuse and varicose and the extracellualr matrix was often filled with fibrous matter.

The alterations in the dermal papilla and the degeneration of dermal papilla cells from follicles of clinically normal scalps suggests that the dermal papilla may be a prime site of damage in the aetieology of alopecia areata.

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EVOLUTIONARY CHANGES OF PERIPHERAL NERVES IN SKIN LESIONS OF ATOPIC DERMATITIS. Hisashi Sugiura, Mitsuyoshi Omoto, Kiichiro Danno, Masami Uehara, Department of Dermatology, Shiga University of Medical Science, Otsu, Japan.

Although increased number of substans P positive nerve fibers is reported in skin lesions of atopic dermatitis, a quantitative analysis of peripheral nerve fibers has not been described in the disease. We then examined a degree of dermal nerve hyperplasia and a epidermal penetration of free nerve ending in various skin lesions (10 normal appearing skin, 10 early, 20 subacute, 20 lichenified, and 10 prurigo lesions) of atopic dermatitis. A normal skin of 15 non-atopic individuals was similarly examined as controls. Peripheral nerves were stained with a pan-neuronal marker protein gene product 9.5. Area of peripheral nerves was measured by image analyser IBAS-1. Extensive hyperplasia of peripheral nerve fibers were seen in lichenified and prurigo lesions. Normal appearing skin or early lesions of the disease showed normal or mild hyperplasia of the nerves. Epidermal penetration of peripheral nerves was consistantly observed in normal appearing skin of patients with atopic dermatitis and non-atopic individuals. The number of free nerve endings penetrating into epidermis was decreased in subacute skin lesions of atopic dermatitis compared to normal appearing skin. From these findings it is possible that the increased number of peripheral nerve fibers and epidermal free nerve endings in skin lesions of atopic dermatitis may play a role in provoking or aggravating the itchiness of atopic dermatitis.

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MORPHOLOGICAL CHARACTERISTICS OF *STAPHYLOCOCCUS AUREUS* CELLS COLONIZING ON THE SKIN SURFACES IN ATOPIC DERMATITIS.

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The pathogenesis of atopic dermatitis is complex and remains poorly understood. Normal as well as diseased skin of patients with atopic dermatitis is much more densely colonized with *Staphylococcus aureus* compared to normal skin and other types of eczematous skin. Such severe *S. aureus* colonization in atopic dermatitis has been suggested to play an important role in the development and/or exacerbation of the dermatitis. Gram-stained stamps from wet eczematous skin surfaces of 10 patients with severe atopic dermatitis were examined in correlation with migrated blood cells. Scrub or biopsy specimens from eroded eczematous lesions of three patients were processed for electron and immunoelectron microscopy. Ultrathin sections embedded in Lowicryl were incubated with primary antibodies against protein A, fibronectin, fibrinogen, human IgG, and type IV collagen, and with second antibodies conjugated with colloidal gold. The number of *S. aureus* cells on the skin surfaces was varied in each sample, and phagocytosis of *S. aureus* cells by neutrophils was rare. Electron microscopy demonstrated clusters of *S. aureus* cells were present between horny cells or attached on the uppermost horny cells. An intense immunogold labeling for IgG and fibrinogen was uniformly found not only all over the surfaces of *S. aureus* cells but in the intercellular spaces of the epidermis. There was a similar labeling pattern for type IV collagen although no labeling was revealed in intercellular spaces. A moderate labeling for fibronectin was also demonstrated on *S. aureus* cells, but its labeling was partial on each cell. The labeling for protein A depended on samples. These findings indicate 1) *S. aureus* cells multiply on and between horny cells of eczematous skin, 2) plasma components such as immunoglobulins and fibrinogen play important roles in colonization of *S. aureus*. Furthermore, some toxins derived from *S. aureus* should be more investigated in correlation with exacerbation of atopic dermatitis.

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Chronic Dermatosis Therapy Applied to Patients Affected by Chernobyl Tragedy

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Chronic dermatosis therapy (psoriasis - 29 patients, acne disease - 85 patients, focal neurodermite - 9 patients) was carried out by means of cryo-ultrasonic treatment in combination with immuno- and ointment therapy. Full recovery was registered in 66 cases, major improvements - in 42 cases. Best results of the above mentioned therapy were achieved in cases of focal neurodermite and acne disease.

It is worth mentioning that alongside with considerable improvements of skin condition its immunological blood quotients have been normalized as well as supplying skin with oxygen.

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SELECTIVE APOPTOSIS OF MELANOCYTES AS A MANIFESTATION OF BORRELIA BURGDORFERI AFFINITY TO THE NEUROECTODERM IN ERYTHEMA MIGRANS.
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14 cases of seropositive Erythema migrans (5 of them confirmed in cultivation) were studied under the electron microscope. The biopsies were taken from the periphery of the exanthema. The dominant finding was: scattered unicellular necrobiosis (apoptosis) of dendritic cells among basal and suprabasal keratinocytes. In fibrillar metamorphosis of their cytoplasma and nucleus there were solitary melanosomes (1-2) and some incospicious rests of the rough endoplasmic reticulum. The keratinocytes in contact contained some medium-sized vacuoles mainly round their nuclea. But the main part of the keratinocytes were not damaged. Langerhans cells were well equiped containing 4-15 Birbeck granules of rod shape and only scarcely some of them had 1-2 racquet shaped granules. Lymphocytes within the epidermis were rare, their cytoplasma was displaced by big emty vacuoles which deformed the shape of the cell and once there was found a centriol. These results suggest: a) probable affinity of the Borrelia burgdorferi in epidermis to the neuroectodermal structures; b) the delayed immune reaction is somehow disabled by damaged both Langerhans cells and T-cells.

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AN ELECTRON MICROSCOPIC STUDY OF KERATINOCYTE DAMAGE AND THE EPIDERMAL INFILTRATE IN HERPES ZOSTER.
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The purpose of this study was to investigate morphologic aspects of lesional keratinocytes (KC) and the epidermal infiltrate at various stages in herpes zoster (HZ) by scanning (SEM) and transmission electron microscopy (TEM). When erythematous lesions were visualized by TEM, many of KC showed evidence of cell damage. These were characterized by intracellular edema, disappearance of desmosomes, and the widening of intercellular space (ICS). In these cells, complete varicella-zoster (VZ) virus particles were observed in the portion of cytoplasm and in the nuclei. Macrophages and/or lymphocytes migrated through the ICS of degenerated KC. In the early vesicular lesions, we observed by SEM that some infiltrating cells directly adhered to epidermal KC. TEM showed that the morphologic characteristics of adhered cells were identical to those of neutrophils. The KC disclosed the swelling of nuclei and the irregular clumping of chromatin. They filled with numerous VZ virus within the cytoplasm. In the late vesicular stage, KC with balloon-degenerated and surface-cracked configurations were observed in the cavity. Virus particles were located ICS as well as in the cytoplasm of the infected KC. In the pustular stage, ruptured and cytolytic KC with numerous neutrophils were observed in the reticular-degenerated epidermal tissue. These observations suggest that in HZ the epidermal damage may due to cellular response as well as the viral infection. The mononuclear cells in the early stage may predominantly act to initiate viral antigens. Neutrophils in the latter stage may infiltrate as effector and scavenging cells in the infected epidermal tissue.

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OBSERVATION OF TREPONEMA PALLIDUM BY USING SCANNING AND TRANSMISSION ELECTRON MICROSOPES.
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Morphoiogic analysis of *Treponema pallidum(T. pallidum)* was carried out through scanning and transmission electron microsopes. Under the electron microsopes, each *T. pallidum* appeared 7 to 12 treponemas. The frequency wave of the *T. pallidum* was 0.6 to 1.2 microns and the ampliitude of *T. pallidum* was 7.0 to 13.0 microns, and the body-axis was 8.3 to 22.0 microns, and the body-axis 1:1.2 to 1.9. The cell wall of *T. pallidum* was consisted by two layers. The outer layer had high electron density and the inner layer had low density. Cytoplasma was surrounded by cell membrane. The transverse diameter was about 0.16 micron. The sides of *T. pallidum* frequently became narrow and appeared circular cone . The transverse diameter of apex was about 0.08 micron. Propagation of *T. pallidum* appeared to be transverse departure and multiplying . *T. pallidum* adhered to host's cell in tissue . That is keratinocytes , langhans' cells , endothelial cells , plasma cells and fibroblastes . The cone side of *T. pallidum* adhered to host's cell and dissolved host cell membrane, and there were many different sizeholes on the surface of host's cell . Inside the feeze-fractural host cytoplasmas there were *T. pallidum* which were propagting and parasiting. It was showed "space phenoucenon" between *T. pallidum* and host cytoplnsm. This article is aimed to study the uitrastructure of *T. pallidum* , combinning with the pathologic changes of syphilic chancre and condylomata lata lesion induced by adherence of *T. pallidum* to host's cell appeared in preliminary syphilis.

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LOCATIONS WHERE LYME BORRELIOSIS SERA (IgG) REACT ON THE JAPANESE BORRELIA BURGDORFERI (YT-2).
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Antigen of Lyme borrelia varied between strains from different countries. However, antigens obtained from strains of western countries are used for serological tests. This study was undertaken to clarify the location where sera of Japanese Lyme borreliosis patients react on the Japanese *Borrelia burgdorferi* (YT-2). Four positive sera of Lyme borreliosis and 1 negative serum for control after tick bites were used for immuno-electron microscopy. YT-2, derived from *Ixodes persulcatus*, were fixed and embedded in a low temperature polymerizing water soluble resin (Lowicryl) a t-20℃. IgG reacted to YT-2 on ultrathin sections were detected with 5 nm gold particle-labeled anti-human IgG goat serum. Lyme borrelia from U.S.A. (B-31) was also use for comparison. Gold particles were mainly on outer envelope, cytoplasm and flagella, and less on blebs with positive sera. Sera showed different assortment of these parts of YT-2 in these various cases. Negative serum showed no accumulation of particles on YT-2. There were no remarkable differences between patterns of reacted sites with YT-2 and B-31. These findings suggest that sera detected portions are different for each case and serological tests containing whole antigen of Lyme borrelia will be more useful for screening. Similar results will be found in tests with either YT-2 or B-31.

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FACTOR XIIIa POSITIVE CELLS IN NORMAL PERIPHERAL NERVES AND CUTANEOUS NEUROFIBROMAS OF TYPE1 NEUROFIBROMATOSIS.
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The histogenesis of cutaneous neurofibromas, particularly the origin and nature of fibroblast-like cells, is controversial. Recently, cells containing blood coagulation factor XIIIa have been reported in cutaneous neurofibromas, though their identity is uncertain. In the present report, by the combination of double immunohistochemical staining and immunoelectron microscopy, we demonstrate that factor XIIIa positive cells in cutaneous neurofibromas are phenotypically and morphologically distinct from Schwann cells, perineurial cells, endothelial cells, mast cells and conventional macrophages, but correspond to fibroblast-like cells. However, the factor XIIIa positive cells in cutaneous neurofibromas differ from conventional fibroblasts in the strong expression of HLA-DR antigen and lack of a fibroblast marker. On the other hand, endoneurial fibroblasts and occasional connective tissue cells within perineurium and epineurium in normal peripheral nerve fibers express factor XIIIa as well as HLA-DR antigen. The results suggest that fibroblast-like cells in cutaneous neurofibromas are most likely derived from factor XIIIa positive and HLA-DR antigen positive connective tissue cells in peripheral nerves. The role of the factor XIIIa positive cells in the growth and development of cutaneous neurofibromas will be discussed.

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ARGYROPHILIC NUCLEOLAR ORGANIZER REGIONS (AgNORs) IN NEVOMELANOCYTES AND KERATINOCYTES OF SPITZ NEVI AND MALIGNANT MELANOMAS:
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One of the most difficult problems in dermatopathology represents the differentiation of a benign Spitz's nevus (SN) from malignant melanoma (MM). Argyrophilic nucleolar organizer regions (AgNORs) are loops of DNA which carry the genes for ribosomal RNA and for associated proteins. The exact function of these proteins is still unclear but they are supposed to exhibit regulatory functions in controlling the genes for ribosomal RNA and are therefore involved in malignant cell proliferation. In order to investigate whether determination of AgNORs is a useful tool to differentiate SN from MM a silver staining technique for the identification of AgNORs was applied to routinely processed paraffin sections of 14 SN and 14 MM. In all MM cases the histological diagnosis was confirmed by the fact that the patients developed metastases within an observation period of 5 years. AgNOR staining was performed according to established procedures and AgNORs were counted by light microscopy. In each lesion AgNORs were quantified in nevocellular and melanoma cells (NCC/MC), epilesional epidermal cells (EEC) and perilesional epidermal cells (PEC). All statistical analyses were performed as two-tailed tests and p-values < 0.05 were considered as statistically significant. Mean AgNOR counts of NCC/MC did not differ statistically significant between SN and MM (4.1 SD +/- 1.3 vs. 4.3 SD +/- 1.5; 2p = n.s.). EEC above MM exhibited a higher AgNOR count than EEC above SN (6.07 SD +/- 1.81 vs. 4.98 SD +/- 1.22; 2p = n.s.). A significant correlation was found between EEC and NCC/MC in MM ($r = 0.71$, $p = 0.03$) but not between EEC and NCC in SN. In conclusion, our data indicate that the direct quantitation of AgNORs in NCC/MC is without value for the differentiation of SN from MM. However, it seems very likely that epilesional epidermal cells are stimulated in AgNOR expression by MM and therefor MM but not SN are secreting factors which stimulate proliferation activity of epidermal cells.

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COMPUTER ASSISTED ESTIMATION OF THE VOLUME WEIGHTED MEAN NUCLEAR VOLUME DISCRIMINATES SPITZ'S NEVI FROM MALIGNANT MELANOMAS. Andreas Steiner, Michael Binder, Ulrike Mossbacher, Klaus Wolff, Hubert Pehamberger, Department of Dermatology, University of Vienna Medical School, Vienna, Austria

Spitz's nevi are benign melanocytic skin tumors which are usually differentiated from malignant melanomas by histopathological criteria. Often, however, the architectural pattern and cytologic features of Spitz's nevi and melanomas are similar and thus Spitz's nevi may be confused with malignant melanomas at the histopathological level. The determination of volume weighted mean nuclear volume (Vv) employs a technique which permits an unbiased and efficient estimation of nuclear volumes in tissues, particularly in tumors. In this study volume weighted mean nuclear volume was determined in 13 Spitz's nevi and 14 malignant melanomas in order to investigate whether this stereological approach may be of use in the differentiation of these two tumors. Vv was determined by computer assisted image analysis (IBAS 2C, Kontron, Germany) on Feulgen stained sections employing sterological estimation of the volume weighted mean nuclear volume. The mean Vv (+/- SD) of Spitz's nevi was 491.6 μm^3 (SD +/- 175.1), whereas malignant melanomas exhibit a significantly higher ($p<0.001$) Vv of 775.2 μm^3 (SD +/- 205.4). This difference was even more pronounced when the deeper portions of the lesions (Spitz's nevi: 443.1 μm^3, SD +/- 142.4; malignant melanomas: 864.1, SD +/- 169.6) were investigated. In addition, in relation to the depth of the lesions the mean Vv decreased in Spitz's nevi whereas it increased in melanomas. We found that (1) malignant melanomas reveal a larger Vv than Spitz's nevi in general, and (2) in contrast to malignant melanomas, the Vv of nevomelanocytes in Spitz's nevi decreases in the deeper portions of the dermis. Vv may be regarded as helpful tool for the differential diagnosis of Spitz's nevi and malignant melanomas.

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A MODE OF TUMOR CELL PROLIFERATION IN A CUTANEOUS TYPE OF ATLL. Masayoshi Johno, Munashi Ohishi and Tomomichi Ono. Department of Dermatology, Kumamoto University School of Medicine, Kumamoto, Japan

Some cases of adult T-cell leukemia/lymphoma(ATLL) seem to display clinical and histological signs indistinguishable from mycosis fungoides(MF); erythematous plaques for the initial 1-2 years, followed by cutaneous tumor and systemic malignancy. We analyzed twelve such cases of ATLL by molecular biology and immunohistochemistry. In cutaneous lesions at the erythematous plaque stage, Pautrier's micro-abscesses(PMs) were present in all cases. Large tumor cells were abundant in the epidermis and papillary dermis. Immunohistochemically, about 95%, 80% and 35% of the tumor cells were positive for CD25, proliferation-related antigens and BrdU incorporation, respectively. Interestingly, almost all of the macrophages and Langerhans cells in the PMs and papillary dermis lost a HLA-DQ antigen, while HLA-DR,-DP and CD1a antigens remained. By southern blot analysis, monoclonal integration of human T-lymphotropic virus type-1 (HTLV-1)proviral DNA was demonstrated in all biopsy specimens of the skin lesions. These findings suggest that the epidermis and papillary dermis are major sites for ATLL cell proliferation, and that the proliferation is regulated by cytokines produced by epidermal cells, and by a DQ-related mechanism in the early stage of MF-like cutaneous ATLL.

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PHENOTYPIC HETEROGENEITY IN LANGERHANS CELL HISTIOCYTOSIS

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Langerhans cell histiocytosis (LCH) is a disorder in which Langerhans cells (LC) are the predominant proliferating cells. The immunophenotypic and ultrastructural charateristics of LC in LCH are virtually identical to those of normal LC. However, data on the monocytic/macrophage differentiation of LC in LCH were increasingly reported and immunophenotypic heterogeneities were described. In this study, we demonstrate further evidence for the heterogenous phenotype of LC in LCH. We used a panel of monoclonal antibodies racting with monocyte/macrophage-associated antigens, e.g. Ki-M1p, PGM-1(CD68R) and a mAb associated with cutaneous lymphocytes (CLA), HECA-452, which is additionally reative with HEV in lymphoid tissues, some monocytes and - to a certain extent - with normal epidermal LC. We studied 53 biopsies from different organs (localized + disseminated LCH) on paraffin sections, applying a 3-step immunoperoxidase technique. Irrespective of the clinical presentation or the type of inuvolved organ, virtually all cells were S100+, CD 45+ and HLA-DR+. Ki-M1p and PGM-1 positivity was seen in all biopsies, in a range from 10 - >90%. Giant cells and small-sized LC showed uniformely strong, granular intracytoplasmatic staining, whereas medium-sized LC failed to react with both mAbs in most cases. We could not detect Ki-M1p+ or PGM-1+ "normal resting" LC in control biopsies of normal skin, whereas HECA-452 mAb was shown to react with many, but not all epidermal LC. In LCH, the proliferating cells showed similar features: HECA-452+ LC were detected in all biopsies, (range 5->90% prolif. LC). Skin biopsies (n= 12), with strong epidermotropism of infiltrating LC and formations of subcorneal abscesses, showed the most pronounced HECA-452 positivity (range 50->90% HECA-452+LC). In other organs (bone, liver, lymph node or lung) HECA-452+LC were predominantely located in areas with accumulation of eosinophilic granulocytes. Ag-expression was seen focally on up to >90% of LC, whereas areas without eosinophils showed only single HECA-452+LC. Giant cells failed to stain with HECA-452 in all biopsies. This study provides further evidence for the heterogeneity of LCH. The capacity of LC in LCH to vary immunophenotypically (from the phenotype of normal LC to LC with monocytic/macrophage differentiation phenotype) supports the concept of LCH as a proliferative (non-neoplastic) disorder of LC. We could demonstrate that LC, proliferating within and outside the skin, express the HECA-452 antigen, possibly due to cytokine modulation and/or induction by cells (eosinophilic granulocytes?) in the environment of proliferating LC.

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SEBOCRINE ADENOMA: CLINICAL, LIGHT, AND ELECTRON MICROSCOPIC FINDINGS IN A SERIES OF THIRTY CASES. M. Tarif Zaim and Madhat Hasan, Departments of Dermatology and Pathology, University Hospitals of Cleveland and the Veterans Administration Medical Center, Cleveland, Ohio, U.S.A.

Sebocrine adenoma is a poorly-understood benign cutaneous appendageal neoplasm with sebaceous and apocrine differentiation. Reported herein are thirty examples accumulated since our first description of this neoplasm (Am J Dermatopathol 10:311-318, 1988). Clinically, the tumor manifests as a flesh-colored papule. Histologically, the tumor shows areas that are eccrine poroma-like, with anastomosing broad bands of small uniform polygonal cells interspersed by cuboidal epithelium-lined ducts and cystic spaces filled with amorphous material. Unlike eccrine poroma, solitary, and/or clustered sebocytes, and granular layer-lined ducts (or so-called squamous eddies) are also seen throughout the tumor. Ultrastructurally, ducts with broad cuticular border, luminal cells with stumpy microvilli, and mostly, clear outer cells are representative of the poromatous areas of the tumor. These cells are connected with interdigitating microvilli, and contain small amounts of tonofilaments. Partially-differentiated and mature sebocytes are also present. The association of apocrine and sebaceous elements in the same tumor lends support to a sebaceous-apocrine differentiation rather than an eccrine differentiation, for it recapitulates the normal development of the pilosebaceous-apocrine unit.

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DF3(CA15-3) MONOCLONAL ANTIBODY AS A MARKER OF CUTANEOUS ADNEXAL TUMORS. Shyoko Yasui[1], Iwao Ando[1], Atsushi Kukita[1], Haruko Hino[2], Department of Dermatology, 1. Teikyo University Mizonokuchi Hospital, Kawasaki, Japan. 2 Kantohchuoh Hospital, Tokyo, Japan.

Various kinds of antibodies have been studied as markers of adnexal tumors. One of characteristics of skin appendage is epithelial mucin production by secretory segment of sweat gland and sebaceous gland. But the epithelial mucin distribution in adnexal tumors is not well studied yet. We characterize the cutaneous adnexal tumors by the monoclonal antibody DF3 which detect the 20 amino acids sequence in the core protein of epithelial mucin. Tumors are taken from patients visited our hospital or Toranomon hospital, fixed in buffered fromalin, embedded in paraffen and sectioned. Then, they are stained by DF3 monoclonal antibody with immunoperoxidase method. The results are all epidermal tumors and syringoma are DF3 negative. Lumina margins of mixed cell tumor, eccrine spiradenoma, syringocystadenoma papilliferm etc. are DF3 positive. Not only lumen but some solid parts of eccrine poroma and clear cell hidradenoma are DF3 positive. Eccrine porocarcinoma and especially extramammary Paget's disease are DF3 positive without any staining pattern of polarity or lumina structures. DF3 is a useful antibody for characterization fo adnexal tumors. Acknowledgements. We thank Dr. Ohara for several specimens of Toranomon hospital.

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ASSESSMENT OF CELLULAR PROLIFERATION OF ECCRINE ACROSPIROMAS AND ECCRINE GLAND CARCINOMAS. Shin-ichi Ansai, Yutaka Hozumi and Shigeo Kondo, Department of Dermatology, Yamagata University Faculty of Medicine, Yamagata, Japan

The proliferating activity of tumor cells is an important factor reflecting the malignant potential. Recently, the immunostainings using the antibodies to proliferating cell nuclear antigen (PCNA) and Ki-67, and the counting of argyrophil nucleolar organizer regions (AgNORs), have enabled to estimate the activity. In this study, we discuss the utility of these examinations in judging the malignant potential and prognosis of eccrine acrospiroma and eccrine gland carcinoma. In our study, routine processed formalin-fixed and paraffin-embedded tissue specimens surgically excised from 25 cases of eccrine gland carcinoma (7 cases with stage III and 18 cases with stage I or II) and 25 cases of eccrine acrospiroma were used. PCNA and Ki-67 labelling indices were categorized semiquantitatively into four grades. AgNOR counts, and PCNA and Ki-67 labelling indices between eccrine acroapiroma and eccrine gland carcinoma, and between cases with stage III and stage I or II, were compared. Significant differences were found between eccrine gland carcinoma and eccrine acrospiroma with these three parameters ($P < 0.01$). These data indicate that AgNOR counts and semiquantitative grading of PCNA and Ki-67 labelling indices are useful in differentiation between eccrine acrospiroma and eccrine gland carcinoma, while none of these parameters demonstrate a prognostic value in eccrine gland carcinoma.

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ARE SWEAT ORGAN TUMORS DERIVED FROM SWEAT ORGAN? — From a case of coexistence of cylindroma, spiradenoma and trichoepithelioma —

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A 55-year-old woman had developed soybean-sized papules dispersed on the forehead from 10 years earlier. After resection, the lesions recurred. Histomorphological findings on them revealed co-existence of cylindroma and spiradenoma, dispersed immature hair tissues and occurrence of tumors from hair follicles in part. By using anti-keratin antibodies, the tumors showed a multidifferentiation into eccrine, apocrine and sebaceous gland and hair, consistent with the findings on the reactivity of external root sheath. While cylindroma and spiradenoma are considered to be tumors deriving from the sweat organ, the present case suggested the tumors deriving from the sweat organ, the present case suggested the tumors deriving from outer root sheath cells capable of multidifferentiation. Accordingly, there seem to be cases of hair follicle-derived tumors among those considered to have sweat organ tumors. Meanwhile, immunohistological findings on sweat organ tumors obtained at our clinic are also reported.

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p53 PROTEIN EXPRESSION IN MAMMARY AND EXTRAMAMMARY PAGET'S DISEASE.

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p53 is a tumour-suppressor gene, mutations of which can turn it into an oncogene. Whereas the wild type p53 has a short half-life, the mutated p53 protein accumulates within the cell and can thereby be detected by immunohistochemistry. Up till now the p53 protein has been found increased in various (pre)malignant skin lesions. In the present study we studied by immunohistochemistry, using the DO7 monoclonal antibody, the expression of p53 in 16 cases of Paget's disease (11 mammary-MPD and 5 extramammary-EPD), a rare cutaneous malignancy whose histogenesis remains uncertain. Four of the MPD specimens (i.e. 36%) showed specific expression of p53 within Paget's cells; one of these cases comprised an invasive ductal adenocarcinoma in the underlying dermis, and this was also p53-positive. By contrast, all EPD cases proved p53-negative. The surrounding epidermal and adnexal keratinocytes were p53-negative in all cases. The differential pattern of p53 expression between MPD and EPD underlines the difference between these two diseases and suggests that, despite similar morphological characteristics, these may be due to different genetic mechanisms; furthermore our data provide evidence in favour of the origin of mammary Paget's cells from the underlying adenocarcinoma (rather than the surface epithelium).

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MAMMARY AND EXTRAMAMMARY PAGET'S DISEASE: AN IMMUNOCYTOCHEMICAL STUDY USING ANTI-TUMOR ANTIBODIES.

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The pathogenesis of mammary (MPD) and extramammary (EMPD) Paget's disease has remained controversial; and debate has intensified in recent years as data has accumulated from immunocytochemical investigations. In the present study, we used monoclonal antibodies to three different tumor antigens (Ca 15-3, Ca 19-9 and SLX) which had not been used to Paget's disease and compared with each other and CEA.

Surgically removed and formalin-fixed paraffin-embedded tissues from seven mammary, five vulvar, two scrotal, and two axillar Paget's disease were studied for immunocytochemical antigens.

Paget cells stained with the anti-Ca 15-3 antibody. The intensity of the staining was strong. Staining with this antibody was also observed in the ductal and secretory portions of the eccrine and apocrine glands, and in sebaceous gland cells. Staining with anti-Ca 19-9 antibody was mainly observed in the eccrine duct, but not in other skin components. The Paget cells stained with anti-SLX antibody strongly. Staining with this antibody was also observed in the apocrine secretory coils, lactiferous ducts, epidermal dendritic cells, dermal fibroblasts and inflammatory infiltrates.

Immunoreactivity with anti-Ca 15-3 antibody was more reproducible and reliable than that with antibody against CEA because a positive reaction for CEA was weaker than that of Ca 15-3 and was not always found in all Paget cells. When compared with antibody to Ca 15-3, that to SLX was less useful because the latter had immunoreactivity with epidermal dendritic cells and many infiltrating cells in the dermis. Thus, antibody to Ca 15-3 seems most useful among four tumor markers to stain Paget cells.

Depending on the staining pattern of the antibodies it is suggested that the origin of Paget cells is secretory cells of apocrine sweat glands in EMPD, and luminal lactiferous ducts in MPD.

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METABOLIC CHANGES IN PSORIASIS AND PSORIATIC NEPHROPATHY.

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We analysed the role of carbonhydrate, lipid and purine abnormalities in clinical and morphological features of psoriatic nephropathy for three groups of patients namely Gr1 with proteinuria (0.05-3.8 g/l), Gr2 with microalbuminuria and psoriatic patients without proteinuria and microalbuminuria (Gr3). In Gr1 and Gr2 we observed significantly higher levels of serum and urine uric acid, serum triglycerides, total cholesterol, increased (VLDL+LDL)/HDL ratio in comparison with Gr3. Besides that in Gr2 comparing to Gr1 and Gr3 there were significantly increased levels of glycemia and basal glomerular filtration rate (GFR), decreased renal reserve (i.e. GFR response to acute protein load), positive correlation of glycemia and uric acid levels with basal GFR. Morphological study of renal biopsy in Gr1 (34 cases) revealed various types of glomerulonephritis and focal and segmental glomerulosclerosis with LDL-depositions (4 cases) and uric acid crystals (1 case), in Gr2 (8 cases) it revealed thickened glomerularbasement membrane and mesangium proliferation mainly in area of glomerular hilus. The hyperlipidemia with increased (VLDL+LDL)/HDL ratio, uric acid and hyperperfusion due to hyperglycemia and hyperuricemia is supposed to cause morphological changes in Gr1 and Gr2.

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A NEW MODEL FOR CYCLOPHOSPHAMIDE-INDUCED ALOPECIA AND ITS MANIPULATION BY CYCLOSPORINE A AND TOPICAL DEXAMETHASONE.

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Alopecia as a side-effect of chemotherapy remains a major problem in clinical oncology. Reliable screening systems for the detection of effective means to block the development of this alopecia are required. Here, we introduce cyclophosphamide (CYP)-induced alopecia in the C 57 BL-6 mouse as one particularly attractive model. It assesses the effects of a single intraperitoneal injection of CYP (100-150 mg/kg) on the growth, pigmentation and recovery of mature, depilation-induced anagen VI hair follicles in adolescent animals. This was followed over time and analyzed by assessing hair cycle-dependent changes in skin color, by histological morphometry and by electron microscopy. CYP-treated mice display macroscopic and histological phenomena that strikingly resemble the characteristic hair follicle pathology seen with CYP-induced alopecia in man: patchy alopecia of rapid onset, melanin clumping and incontinence, dermal papilla swelling, and distension of the follicular canal. Also, the pattern of follicle recovery and repigmentation follows that occuring under clinical conditions. Anagen VI follicles respond to CYP by the induction of dystrophic anagen and catagen, and there are regional differences in the follicular vulnerability to CYP. Ultrastructurally, cell damage to hair matrix keratinocytes and dermal papilla fibroblasts and extracellularly located, abnormal melanosomes were seen in CYP-treated mice. CYP-induced alopecia could be attenuated by intraperitoneal application of cyclosporine A (3 x 5 mg/mouse) and enhanced by topical dexamethasone (0.1%). Due to its similarity with CYP-induced alopecia in man, this is the clinically most relevant animal model available to-date for studying the pathobiology of chemotherapy-induced alopecia and for screening for drugs that modulate this alopecia.

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EVIDENCES OF PERCUTANEOUS ABSORPTION OF A NOVEL TOPICAL CYCLOSPORIN A FORMULATION

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Effects of topical cyclosporin A (CsA) on psoriasis and alopecia areata is controversial. Since the intralesional injection of CsA produced a significant resolution of psoriatic plaques, some investigators speculated that the penetration of the drug was a problem of the topical CsA formulation. Recently Shiseido Co. formulated a novel CsA 5% cream. In the present work, we investigated the effect of this formulation on the proliferation of human epidermal keratinocytes grafted onto nude mice by using 5-bromo-2'-deoxyuridine (BrdU) Furthermore, the effect on the hair growth of Balb/c mice was studied. The drug was applied twice a day on the human skin grafted onto anterior dorsal region of nude mice for seven days. As a control, a vehicle was applied. During last two days of the experiment, all mice were injected intraperitoneally with 100mg/Kg of BrdU. Incorporation of BrdU was revealed immunohistochemically. The percentage of positive cells reduced in CsA-treated human grafts in comparison with that of control (CsA: 19.7±1.2%, control: 40.6±12.1%). For the study of the hair growth, CsA was applied twice a day on anterior dorsal region of eight-week-old Balb/c mice. Histologically, anagen hairs increased in number in all skin sections obtained from CsA-treated mice in comparison with that in skin sections obtained from control mice. Since these results were consistent with those demonstrated in studies with systemic administration of CsA, this novel topical formulation may be effective on several skin disorders which improve with oral formulation of CsA.

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EFFECTS OF TOPICALLY APPLIED SPIRONOLACTONE ON ANDROGEN STIMULATED SEBACEOUS GLANDS IN THE HAMSTER PINNA. Taisuke Seki and Masaaki Morohashi. Department of Dermatology, Faculty of Medicine, Toyama Medical and Pharmaceutical University, Japan

Spironolactone is a diuretic antihypertensive drug which acts at the aldosterone-dependent sodium-potassium exchange site in the distal convoluted renal tubule. Spironolactone has been also shown to produce antiandrogenic effects by competitvely inhibiting dihydrotestosterone at the androgen receptor sites. We investigated the effects of topically applied spironolactone on the androgen stimulated sebaceous glands by measuring the post-application size of the sebaceous acinus as an index of the effect using whole mount techniques in adult male golden hamsters.

Topically applied spironolactone induced a 23% reduction of the sebaceous gland size with a significantly higher suppression rate ($p<0.01$) than that of the matching placebo in androgen stimulated hamster pinnae.

Increased sebaceous secretion, one of the various factors in the pathogenesis of acne vulgaris, is induced by the binding of dihydrotestosterone to androgen receptors in the skin. Thus these results suggest potential usefulness of topically applied spironolactone in the treatment of acne vulgaris.

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ELECTRONMICROSCOPIC STUDY OF THE EFFECT OF SHORT-TERM TOPICAL TRETINOIN ON PHOTODAMAGED SKIN. T. Shukuwa[1] and A.M. Kligman[2]. [1]Dept. of Derm., Nagasaki University School of Medicine, Nagasaki, Japan. [2]Dept. of Derm., University of Pennsylvania, Philadelphia, U.S.A.

There have been many reports of the corrective effects of topical tretinoin on photodamaged skin. Previous reports have employed treatment schedules of six months and longer. To determine the improvement obtained after a short-term topical application of tretinoin, five males, ages 52 to 61, with severely photodamaged skin received 0.05% tretinoin cream twice daily for one month to the skin of the dorsal forearm. The following procedures were done before and after treatment: (1) 3 mm punch biopsies for histochemistry and transmission electron microscopy, (2) sticky slides for melanization of corneocytes, (3) detergent scrubs for corneocyte sizing and morphology, (4) cyanoacrylate stripping for scanning and transmission electron microscopy and (5) Silflo replicas for surface topography. The epidermal histologic abnormalities were largely corrected by one month of treatment. Atypia and dysplasia were eliminated accompanied by acanthosis, restoration of polarity and a thinned horny layer. On detergent scrub, the population of corneocyte disaggregated from horny layers was reduced after treatment. Ultrastructurally, there were many villus-like projections(VPs) on the corneocyte before tretinoin. The number of VPs was decreased and desmosomal bodies in stratum corneum increased after treatment. Dermal changes were insignificant. Surface glyphic patterns reappeared, melanin granules within corneocytes were greatly decreased, associated with modest bleaching of pigmented spots. Despite these structural improvements, the corneocytes were greatly swollen in size and irregular in shape, reflecting an abnormal stratum corneum barrier. The particular effects of topical tretinoin on the epidermal abnormalities of photodamaged skin can be discerned after only one month of treatment.

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NOVEL ANTIPSORIATIC STEROIDS MODULATE EXPRESSION OF PROINFLAMMATORY CYTOKINES. Christine Ried, Günter Michel, Axel Beetz, Lajos Kemény, Ralf-Uwe Peter, Thomas Ruzicka; Depts of Dermatology, University of Munich, Germany, University of Szeged, Hungary.

Psoriasis is a common hyperproliferative and inflammatory skin disease. Various cytokines are involved in the induction and maintenance of the inflammatory phenotype. Aberrant overexpression of TNFα and IL-8 in lesional psoriatic skin has been described.

We investigated the effects of the novel antipsoriatic steroid mometasone furoate in comparison with the weak and potent steroids hydrocortisone and betamethasone valerate on the expression of these cytokines in normal human keratinocytes (KC). Cultured foreskin KC were incubated in the presence of increasing concentrations (0.1-100ug/ml) of the steroid compounds with or without previous stimulation with TPA (100ug/ml). Gene expression was monitored by semiquantitative mRNA-PCR after 6 and 24 hrs. incubation.

Pronounced alterations of cytokine gene expression have been observed in keratinocytes treated with antipsoriatic steroids. We postulate that modulation of cytokine synthesis may contribute to the antipsoriatic effects of steroids.

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EFFECT OF A CYTOTOXIC PROSTAGLANDIN, Δ^{12}-PGJ$_2$ ON THE CYTO-SKELETON AND E-CADHERIN EXPRESSION IN TRANSFORMED EPIDERMAL CELLS IN CULTURE. Kouichi Ikai, Masamitsu Yamamoto, Norihisa Matsuyoshi and Masanori Fukushima*, Department of Dermatology, Kyoto University Faculty of Medicine, Kyoto and *Department of Internal Medicine, Aichi Cancer Center, Nagoya, Japan

Cyclopentenone prostaglandins (PGs) such as Δ^{12}-PGJ$_2$ and PGA are potent inhibitors of growth in a variety of cultured cells, including epidermal cells. These PGs, administered at the IC_{50} concentration, induce several specific proteins, mainly the heat shock proteins, leading to cell cycle arrest at the G_1 phase. Under this condition, cyclopentenone PGs also induce a change in the integrity of the cell membrane. We examined the effects of Δ^{12}-PGJ$_2$ on cytoskeletal organization and E-cadherin expression in a transformed human epidermal cell line (HSC-1). Addition of Δ^{12}-PGJ$_2$ at the IC_{50} concentration to HSC-1 cells resulted in the disappearance of actin filaments and the disarrangement of keratin filaments, as visualized by fluorescent labeled phallacidine or immunofluorescence. Immunofluorescence using a monoclonal antibody to human E-cadherin demonstrated that E-cadherin was localized at the cell-cell contact regions in HSC-1 cells. Following a 12-hr-incubation with Δ^{12}-PGJ$_2$, E-cadherin was also detected in a uniform pattern along the cell junctions, although the cell morphology was changed. Immunoblot analysis using this antibody revealed that treatment with Δ^{12}-PGJ$_2$ did not alter the quantity of E-cadherin in HSC-1 cells. These results suggest that the effect of cyclopentenone PGs on cell membrane integrity is related to cytoskeleton damage but not to E-cadherin expression in transformed epidermal cells.

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PROTEIN KINASE C AS PHARMACOLOGIC TARGET OF DRUGS USED IN THE TREATMENT OF ACNE ? Susan M. Toso, Kaleen Kitay, Guy F. Webster, and Lutz Hegemann, Departments of Dermatology, Thomas Jefferson University, Philadelphia, PA, USA, and University of Vienna, Vienna, Austria

In recent years, evidence indicating that protein kinase C (PKC) might be an important pharmacologic target mediating anti-inflammatory and antiproliferative drug action has been obtained. Therefore, in the present study we investigated the effects of various drugs commonly used in the treatment of acne on the activity of purified PKC as well as on PKC-regulated cellular functions of human neutrophils. The tetracycline derivatives, tetracycline, minocycline, and doxycycline, as well as benzoyl peroxide were found to inhibit PKC activity in a dose-dependent manner. In contrast, other drugs such as ciprofloxacin, erythromycin and clindamycin only marginally inhibited PKC activity or were without effect. In our cellular test systems, the tetracycline derivatives inhibited the release of the inflammatory mediator, reactive oxygen species, from neutrophils as well as granuloma-like neutrophil aggregation. The potencies of the tetracyclines in inhibiting these cell functions correlated well with their inhibitory activity on purified PKC. Benzoyl peroxide showed marked cytotoxic effects even at concentrations with were not inhibitory on purified PKC. Ciprofloxacin displayed a slight inhibition of neutrophil functions, whereas erythromycin and clindamycin were without effect. In conclusion, the present data provide first evidence that PKC might serve as an important pharmacologic target of the tetracyclines. The differential action of the drugs on PKC might help to explain their different clinical usefulness in various stages of acne.

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CORRELATIONS BETWEEN RECEPTOR SELECTIVITY AND BIOLOGICAL ACTIVITY OF SYNTHETIC RETINOIDS. U.Reichert, J.M.Bernardon, B.Charpentier, P.Nédoncelle, B.Martin, B.A.Bernard, D.Asselineau, S.Michel, M.C.Lenoir-Viale, C.Delescluse, W.R.Pilgrim, Y.M.Darmon, B.Shroot. CIRD GALDERMA, Sophia-Antipolis, Valbonne, France.

Retinoic acid (RA) and its synthetic derivatives constitute one of the most promising groups of drugs in dermatology today, although their potential is limited by a number of toxic effects. The recent discovery of nuclear RA receptors (RAR α-RAR γ) and related proteins (RXR α- RXR γ) offers an appealing rationale for an approach to improve the benefit/risk ratio by the design of receptor-selective compounds. For this purpose, we have evaluated a series of stable aromatic RA analogues for 1°) their in vitro affinity for recombinant human receptors RAR α, RARβ and RARγ, 2°) their transactivating potential in HeLa cells cotransfected with the appropriate RAR expression vectors and a TRE_3-tk-CAT reporter plasmid, 3°) the induction of plasminogen activator in the murine teratocarcinoma cell line F9, and 4°) the repression of plasma membrane-associated transglutaminase in cultured human keratinocytes.

We found that variation of substituents on the aryl ring have little effect on binding to RAR α but that opening of the naphthoic acid ring, which results in benzoic acid derivatives, improves the affinity for RAR α. Reduced lipophilicity in the central region of the molecule, and particularly the introduction of functions with hydrogen bonding properties potentiates RAR α binding and, when combined with certain aryl substituents, results in RAR α selectivity. On the other hand, binding to RAR β and RAR γ is strongly influenced by para (but not meta)-substituents of the aryl ring: hydrogen bond acceptors in this position increase RAR β selectivity, whilst hydrogen bond donors promote RAR γ selectivity.

Transcriptional activation is more sensitive than in vitro binding, but results essentially in the same receptor selectivity profile, whereas the correlation between selectivity and biological activity in more complex cellular models (F9, keratinocytes) is poor.

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1,24(OH)$_2$-D$_3$ ENCHANCES EXPRESSION OF GM-CSF mRNA AND IL-8 mRNA IN ACTIVATED HUMAN DERMAL MICROVASCULAR ENDOTHELIAL CELLS

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Granulocyte- macrophage-colony-stimulating factor (GM-CSF) together with interleukin (IL)-8 are some of the inflammatory mediators synthesized and secreted by cytokine-activated endothelial cells, and participate in cytokine cascade responsible for attraction of leukocytes at sites of inflammation (1). The growth-stimulatory activity of GM-CSF on human dermal microvascular endothelial cells (HDMEC) suggests, that it may be also involved in mediation of angioproliferation in skin inflammation (2).

The possible influence of antipsoriatic vitamin D$_3$ analoque, 1,24(OH)$_2$-D$_3$ (TV-02, Teijin, Tokyo) to the expression of GM-CSF and IL-8, as cytokines with multiple biological properties, accordingly regulation of inflammation, was determined by reverse transcription followed by polymerase chain reaction (RT-PCR) in IL-1α or β activated SV-40 transfered HDMEC (T-HDMEC). Unstimulated T-HDMEC did not express GM-CSF mRNA or IL-8 mRNA. 5 h incubation of T-HDMEC with IL-1α or IL-1β (100U/ml) resulted a pronounced GM-CSF and IL-8 mRNA expression.

1,24(OH)$_2$-D$_3$ (in a concentration of 10^{-11}-10^{-6} M) dose-dependently inhibited the IL-1 induced GM-CSF mRNA expression of T-HDMEC, but slightly influenced the expression of IL-8.

The antipsoriatic effect of vitamin D$_3$ analoques may partly be explained by their ability to inhibit IL-6 (3) and GM-CSF expression of IL-1 activated HDMEC.

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DIMINISHED CUTANEOUS RESPONSE TO INTRADERMAL NEUROPEPTIDES, COMPOUND 48/80 AND HISTAMINE IN PATIENTS WITH SYMPTOMATIC DERMOGRAPHISM.

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The aetiology of symptomatic dermographism (SD) is poorly understood. Histamine is thought to be important in the pathogenesis of SD,but the trigger for histamine release has not been identified. An IgE-mediated plasma factor has been reported, however in many studies no specific IgE or allergic trigger factor has been isolated. Evidence suggests that secretion of mast cell mediators is at least partially under neuronal control. Peptidergic sensory afferent neurones may release peptides in response to physical and chemical stimuli. Intradermal dose response studies of substance P (SP), compound 48/80 (48/80), histamine (H), calcitonin gene-related peptide (CGRP), endothelin 1 (ET1), neurokinin A (NKA), vasoactive intestinal peptide (VIP), and phosphate buffered saline (Sal) were undertaken in 5 patients with SD and 5 age and sex matched controls. Cutaneous weal and flare responses were measured at the site of injection by planimetry at 2, 10, 20 and 60 minutes and cutaneous blood flow was recorded at the centre of the injection site at corresponding times using laser doppler flowmetry. Flare area was significantly reduced in patients compared to controls with 1µmol/l SP at 2 (p=0.046), 10 (p=0.046), 20 (p=0.018) and 60 (p=0.006) minutes; with 0.5µmol/l SP at 2 (p=0.018) and 10 (p=0.046) minutes; and with 0.25 µmol/l SP at 60 minutes (p=0.09). There were significant reductions in flare area with all concentrations of 48/80 (p=0.003-p=0.046), and with all concentrations of H (p=0.006-p=0.046) at 2, 10, 20 and 60 minutes. No significant differences were seen in flare responses to Sal, ET1, NKA, and VIP; and in erythema with CGRP. No significant differences in cutaneous blood flow between patients and controls were recorded. Significant reductions in weal size compared to controls were seen with all concentrations of SP (p=0.006-0.02); 48/80 (p=0.006-0.02); 2 µmol/l VIP at 2 (p=0.025), 10 (p=0.03) and 20 (p=0.004) minutes; 1µmol/l (p=0.03)and 0.5 µmol/l (p=0.03) VIP at 2 minutes; and with all concentrations of NKA up to 20 minutes (p=0.02-p=0.03). No significant differences were seen in ET1-induced pallor. These data indicate that there is a sensory afferent neuropeptidergic defect in symptomatic dermographism which requires further elucidation.

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LASER DOPPLER FLOWMETRY COMPARISON OF PHARMACODYNAMIC EFFECTS OF CETIRIZINE AND LORATADINE ON HISTAMINE INDUCED SKIN RESPONSE.

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In previous studies, reproducibility of the skin response to histamine administered by intradermal pricking (HP) was established using laser Doppler flowmetry (LDF). Hence the method appeared acceptable for performing *in vivo* evaluation of anti-H1 activity in clinically relevant terms to the dermatologist i.e. at the skin level.

In this study, LDF monitoring of the skin response (expressed as perfusion units above baseline; PU) to histamine (100mg/ml) was performed at 4 cardinal points located at 1 cm from the HP sites on the volar aspect of the forearm of 9 non atopic adults. All subjects gave their written informed consent to participate in a randomized, placebo controlled, double blind study comparing the anti-H1 activity of cetirizine (10mg) or loratadine (10mg). Skin responses to HP were evaluated 1h and 5h after a single oral intake of test compounds with a washout period of 8 days between test sessions. Statistical analysis was by analysis of variance; a profile of treatment related variation was evaluated for significance with Scheffe F-test ($p<0.05$).

As compared with skin responses observed after placebo, LDF readings did not show significant variations 1h after drug intake. However, 5h after drug administration, we recorded the expected change, i.e. a reduction of LDF signal at 1cm from HP sites with a significant difference between cetirizine (87.59± 118.97 PU) and loratadine (463.34± 350.06 PU). As indicated by the 95% confidence limits (from -3.8 to 179 and 194 to 732 PU for cetirizine and loratadine respectively) there was no overlap between skin PU recorded 5h after intake of drugs with significantly lower values after cetirizine ($p<0.05$). Both drugs clearly show a different activity profile at their skin targets.

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STUDY OF CHLORIDE CHANNEL REGULATION IN CUTANEOUS CELLS USING CELL VOLUME IMAGE ANALYSIS OF A SINGLE DISSOCIATED CELL.

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Chloride (Cl) channels are ubiquitous and involved in homeostasis and membrane transport in all living cells. Unfortunately, the study of ionic transport, especially that of ion channels, has been neglected in cutaneous cells such as keratinocyte, fibroblasts, and appendageal cells including the sweat gland cells, hair cells, and sebaceous cells. We hypothesize that ion channels in cutaneous cells are dynamically regulated in both normal and pathological conditions (e.g., spongiotic keratinocyte, effects of cytokines on cutaneous cell membrane, differentiation vs. membrane transport, effect of growth factors, cancerous cells). Although membrane channels can be studied using the patch clamping technique, we propose that a simple cell image analysis of cell volume regulation is equally suitable for the study of Cl channels. Freshly dissociated rhesus sweat clear cells and human keratinocytes were used as model systems. The method is based on the fact that if the cell membrane is made permeable to exogenous K channels with valinomycin, then activation of endogenous Cl channels by agents (Ca, ATP, cAMP, PKC activation, cytokine and growth factors, etc.) should cause cell shrinkage due to outflux of KCl from the cell. In contrast, if the cell membrane is made permeable to gramicidin-derived Na channels, then activation of endogenous Cl channels should cause cell swelling due to an influx of extracellular NaCl. In dissociated human keratinocytes, gramicidin-treated cells swell by 10% in response to 1mM ATP-gamma-S, indicating that ATP is one of the regulators of Cl channels in keratinocytes. In the valinomycin system, we also observed a small delayed decrease in the cell volume in response to 1mM CT-cAMP,suggesting that CFTR (cystic fibrosis tranport regulator)-encoded Cl channels are also present. We therefore conclude that the cell volume analysis is a promising and simple methodology applicable to the study of a wide variety of cutaneous cells in health and disease.

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IN VITRO EVALUATION OF GENETIC PREDISPOSITION TO TOXIC EPIDERMAL NECROLYSIS

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The pathogenesis of hypersensitivity reactions has been hypothesized to be dependent on genetic predisposition involving cell defense mechanism. The aim of the present study was to identify genetic detoxication defects involved in severe cutaneous drug reactions. Lymphocytes of 26 patients (including 17 with Lyell or Stevens-Johnson syndromes) were tested for their susceptibility to reactive metabolites generated from drugs by a microsomal oxidation system.The culprit drugs were sulfonamides or anticonvulsants (respectively 13 and 13 patients). Toxicity of culprit drug reactive metabolites (CDRMs) toward patients lymphocytes (9.5% ± 2.2%) was higher than toward controls (3.5% ± 2.2 %) ($p<0.05$). First relatives of 4 patients with Lyell (3 to sulfonamides, 1 to phenobarbital) were also tested. In each family a relative was more susceptible to CDRMs than controls. In order to precise the detoxication defect involved in sulfonamide and anticonvulsivant reactions, we challenged lymphocytes from 11 patients (7 with hypersensitivity to sulfonamides, and 4 to anticonvulsants) to menadione, formaldehyde, and trichloropropene oxide (TCPO). Menadione induces toxicity by oxigen species. Formaldehyde is detoxified by aldehyde deshydrogenase, oxidase and reductase. TCPO is a potent inhibitor of epoxide hydrolase. After a 2 h incubation with one of these three chemicals, no difference of toxicity was found between patients and controls lymphocytes. In conclusion severe cutaneous reactions, especially Lyell or Stevens-Johnson syndromes to sulfonamides and anticonvulsants may be linked to a constitutional and inherited highly specific defect in the detoxication of CDRMs. Our results show that this genetic defect does not involve epoxide hydrolase, oxigen free radicals and / or aldehyde detoxication pathways.

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THE OCCLUSIVE EFFECTS OF PROTECTIVE GLOVES ON THE BARRIER PROPERTIES OF THE STRATUM CORNEUM.

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The aim of this study has been to characterise the effects of occlusion by gloves on the stratum corneum in terms of its physical and functional properties.

A series of volunteer trials have been carried out, looking at the effect of occlusion by patches of PVC glove material on the stratum corneum. Percorneal permeability was assessed using time to onset of nicotinate induced hyperaemia by measuring laser Doppler blood flow. Impairment of barrier function was assessed by measuring trans-epidermal water loss. Surface roughness was measured using silicone rubber skin surface replicas and a stylus profilometer. Compliance of the stratum corneum was assessed by measuring the change in skin surface profile after application of a linear extension device. Hydration was assessed by measuring skin conductance using a skin conductance meter and the water sorption-desorption test.

We found there is at least a short term increase in percorneal permeability, measured as a 38% reduction in time to onset of nicotinate induced hyperaemia; and a temporary impairment of barrier function, measured as an increase in trans-epidermal water loss of 2.2 $gm^{-2}h^{-1}$. We found a temporary reduction in stratum corneum surface roughness and skin compliance, and a temporary increase in basic hydration as measured by skin conductance. However even after baseline conductance measurements have returned to normal levels, we found that peak conductivity after water sorption is still significantly elevated. Peak conductance after water sorption has been related to the ability of the stratum corneum to take up water. This effect was observed up to 3 hours after patch removal.

On the basis of these results we carried out a volunteer study looking at the effects of wearing PVC gloves on stratum corneum barrier properties on the dorsum of the hand. The regimen length was two days. Our results show that there are the beginnings of a cumulative effect. Measurements of TEWL remained elevated by 1.5 $gm^{-2}h^{-1}$ the day after removal of an occlusive glove.

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EFFECT OF GINSENG SAPONINS ON THE GROWTH OF CULTURED HAMAN KERATINOCYTE AND MELANOCYTE. Nack-in Kim, Tae-jin Yoon, Jae-kyung Park, Choong-rim Haw, Department of Dermatology, College of Medicine, Kyung Hee University, Seoul, Korea

The antiproliferative effect on the cultured Keratinocyte (KC) and Melanocyte (MC) with panax ginseng saponins from red ginseng were investigated. Purified ginseng saponins, such as total saponin (TS), panaxadiol saponin : ginsenoside Rb1, panaxatriol saponin : ginsenoside Rg1, and ginseng water extract were provided by Korea Ginseng and Tobacco Research Institute.

Epidermal KC and MC were isolated from the neonatal foreskin and cultured using MCDB 153 and modified TIC media, respectively. And also we try to make the Living Skin Equivalent (LSE) using human fibroblast and bovine collagen. After then various concentrations of ginseng saponins and ginseng water extract were added to each culture system and LSE.

The effects on the cell proliferation were evaluated by inverted microscope and survival cells were calculated using hemocytometer. Ginseng saponins treated LSE were stained with hematoxylin-eosin and Masson-trichrome. Epidermal thickness was observed under the light microscope.

The results were as follows : First, Ginseng saponins and ginseng water extract inhibited proliferation of the cultured KC in a dose-dependent manner. Second, there were no effects on the proliferation or melanization process of the melanocyte. Third, epidermal thickness of the LSE revealed that ginseng saponin treated groups were reduced than control subject. Fourth, apparent cytotoxic effects on the KC were observed in the panaxatriol with concentration of 100 μg/ml or above dosage. This study suggested that ginseng saponins from red ginseng may play a role in the treatment of hyperproliferative skin disease.

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THE EFFECT OF TERBINAFIN IN SOME EXTENSIVE DERMATOMYCOSES. A.L.Mashkilleyson, M.A.Gomberg, Department of Dermatology, Semashko Moscow Medical School, Moscow, Russia

Dermatomycoses (onichomycoses, **Tinea pedis, corporis, cruris**) are among the most common infections in humans. We have studied the effect of terbinafin (Lamisil) in patients with some extensive dermatomycoses including those with acquired immunodeficiency. **Trichophyton rubrum** was found to be the main ethiologic agent isolated from the lesions of our patients. Terbinafin was administered orally in a dose of 250 mg (1 tablet) daily. Bacterioscopy and bacteriologic tests were screened every 2-4 weeks. Patients with **Tinea corporis, pedis** and **cruris** were bacteriologically cured in 2 weeks of the therapy; the average times of bacteriologic cure in onichomycoses of the finger and toe nails were 4 and 8 weeks, respectively, including patients with immunodeficiency. The rate of growth of healthy nails was markedly increased if terbinafin was combined with pentoxifylline (Trental) - the drug enhancing microcirculation. Terbinafin is highly effective in dermatomycoses even in immunodeficiency.

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IN VITRO STUDIES ON THE SIMULTANEOUS PERCUTANEOUS ABSORPTION OF ZINC AND COPPER THROUGH NORMAL HUMAN SKIN TREATED WITH ZINC SULFATE (5%) AND COPPER SULFATE (5%) IN PETROLATUM. Fabrice Pirot, Frédérique Panisset, Annie Rochefort, Philippe Humbert, Pierre Agache, Department of Dermatology, Hospital St. Jacques, Place St. Jacques, 25030 Besançon Cedex.

Zinc and copper are the most widely used of metals in dermatology. Zinc sulfate and copper sulfate are used topically in aqueous solutions and emulsions to restore the healing of open wounds [1].

The pharmakokinetic behaviours of zinc sulfate (5%) and copper sulfate (5%) in petrolatum applied as a single dose to dermatomed abdominal skin of 45 old woman were studied in vitro for 72 hours. The thickness of dermatomed human skin was evaluated by B scanning imaging at 25 MHz (410 μm). A diffusion apparatus with six glass cylindrical cells (3.14 cm^2; 9 ml) was used. The receptor solution was composed of human albumine (5%), 5000 UI penicillin G, 5 mg of streptomycin, 1.25 mg of amphotericin B in physiologic serum (0.9% NaCl). The receptor solution contained 0.775 ± 0.025 zinc mg/l and 0.605 ± 0.005 copper mg/l. The pH of receptor solution was 6.91 ± 0.01. The receptor solution was withdrawn completely after 1.5, 6, 12, 24, 48 and 72 hours for zinc, copper and pH determinations. Zinc and copper were determined by flame atomic absorption spectrometry.

The mean release rate of zinc and copper to the skin were respectively 0.089 ± 0.852 $\mu g/cm^2/h$ and 0.052 ± 0.763 $\mu g/cm^2/h$. The mean of the ratio of the cumulated zinc and copper concentrations was 2.53 ± 0.50. The permeability constant of zinc and copper were respectively 1.05 ± 10.2 x 10^{-7} $cm.h^{-1}$ and 0.63 ± 9.30 x 10^{-7} $cm.h^{-1}$. The diffusion constant of zinc and copper were respectively 9.3 ± 0.56 x 10^{-4} $cm^2.h^{-1}$ and 2.8 ± 0.56 x 10^{-4} $cm^2.h^{-1}$. The pH of receptor solution was not significantly modified for all experiments.

Earlier studies have demonstrated that topical zinc oxide penetrated intact human skin. The mean release rate of zinc to the skin was 5 $\mu g/cm^2/h$ [2]. The flux of zinc sulfate is apparently less important than that of zinc oxide. Interaction of zinc sulfate and copper sulfate and their different biodisponibilities probably explain this difference.

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IMMUNOHISTOCHEMICAL STUDIES ON A STEM CELL ZONE OF HAIR FOLLICLE OF THE HUMAN SCALP. Motohide Takahama. Department of Pathology, Saitama Medical School, Saitama, Japan

Stem cells of the hair follicle are defined as undifferentiated and slow-cycling cells, locating at the zone involving the attachment site of the arector pili muscle. They act as a self-renewing bipotential progenitor compartment committed either to the formation of the hair follicle or to regeneration of the epidermis. Immunohistochemical studies were made to depict the stem cell zone of the hair follicle of human scalp. Human scalp tissues of 60 autopsy cases were formalin -fixed and studied histologically. A large number of serial-sections were made from the selected paraffin blocks and processed to the following immunohistochemical stainings. 1)Proliferating cell nuclear antigen(PCNA) to study S-phase nuclei, 2)tenascin to study embryonic nature, 3)CD34 and 4)HLA-DR; markers for human hematopoietic progenitor and stem cell, 5)cytokeratin PCR-102 made by squamous cell carcinoma cell line. The basal epithelial cells of the "stem cell zone" of the hair follicles expressed negative reaction on PCNA, and positive on the others. The results are suggestive of the unique nature of this zone.

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FIRST PREPARATION OF HEN EGG YOLK ANTIBODIES AGAINST HUMAN HAIR SHAFT AND ITS IMMUNOLOGICAL CHARACTERISTICS. Hiroshi Nojiri, Sachio Naito and Genji Imokawa, Kao Biological Science Laboratories, Tochigi, Japan

Several studies previously demonstrated the immunohistochemical localization of hair structural proteins with antibodies raised against extracted hair proteins, focusing on living to partially keratinized cells. In order to understand the immunological characteristics of fully keratinized hair shaft in relation to keratinization, we attempted to prepare antibodies which can recognize higher-order structures of hair shaft proteins, by using fine hair powders as antigen in stead of extracted or purified hair protein. Hair fine powders were obtained from 11M LiBr-treated swollen hair by frozen pounding without occuring any denaturation of proteins. Because of their low immunogenicity to mammals, they were immunized to hens and polyclonal antibody was purified from egg yolks laid by immunized hens. Immunohistochemical studies with anti-hair powder antibody demonstrated that it had no capacity of reacting with basal cells, but specifically bound to hair cells in the keratogeneous zone and hardened hair cortex. By contrast, typical monoclonal antibody against hair type I keratins stained with only keratogeneous zone, but not with hardened hair shaft. Immunoblot analysis revealed that the yolk antibody recognized type II, high-molecular weight hard keratins. These findings suggest that anti-hair shaft antibodies are useful tool for elucidating the detailed structural changes of hair proteins during the keratinization process of human hair.

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SCANNING ELECTRON MICROSCOPIC OBSERVATIONS OF EXTRACTED HUMAN TERMINAL AND VELLUS HAIR FOLLICLES—SPECIAL REFERENCES TO THE BULGE AREAS. Yutaka Narisawa, Ken Hashimoto and Hiromu Kohda, Division of Dermatology, Department of Internal Medicine, Saga Medical School, Saga, Japan(YN,HK), Department of Dermatology, Wayne State Univ., Detroit, MI(KH)

The bulge area of the hair follicle has recently become a subject of experimental research because of the bulge-activation hypothesis of hair cycle by Cotsarelis et al. and basic structural studies of the bulge seem to be important. Scanning electron microscopy of human terminal and vellus hair follicles were performed on EDTA treated extracted hair follicles which preserved basal cell surface of the outer root sheath in most part. There were many morphological variations of bulge areas such as villous projections in terminal hair follicles and knob like swellings in vellus hair follicles. Moreover, the epithelial hood at sebaceous gland level showed most commonly skirt like structure but variations were also noticed; these were bamboo joints, tulip flower, and long apron configurations. The functional significance of these villi or knob is still obscure, however, they may serve as anchoring points of branched ends of arrector muscles.

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DILATED ROUGH ENDOPLASMIC RETICULUM, ABNORMAL DISTRIBUTION OF COLLAGEN FIBRIL DIAMETERS AND GENE ABNORMALITIES AS CLUES TO THE DIAGNOSIS AND CHARACTERIZATION OF EHLERS-DANLOS TYPE IV. J.D. Marden, H. Kuivaniemi*, J.C. Pierson, J. McMahon**, T.N. Helm**, W.F. Bergfeld and G. Tromp*, Departments of Dermatology and **Pathology, Cleveland Clinic Foundation, Cleveland, OH and *Department of Biochemistry and Molecular Biology, Jefferson Medical College, Philadelphia PA

We report a novel exon splicing defect in the gene for type III procollagen and ultrastructural findings from a 14 year old male product of an incestuous relationship who has a personal and family history spontaneous bowel perforation and joint hypermobility. Ultrastructural examination and type III procollagen analysis were performed to characterize our patient's disorder. Routine histologic studies revealed fibroblasts with markedly dilated rough endoplasmic reticulum as well as normal appearing fibroblasts. Of twenty-five closely examined cells, seventeen showed dilated rough endoplasmic reticulum with cisternal contents filling greater than 75% of cytoplasmic volume in some cells. Abnormal appearing collagen fibrils were also noted, but overall collagen bundle morphology was unremarkable. Analysis of mRNA for the allele of the gene for type III procollagen, obtained from fibroblast cultures, revealed a deletion of 54nt in one allele. The deleted sequences correspond to those of exon 34 and are probably deleted due to a splicing defect. We provide clues to the ultrastructural diagnosis of Ehlers-Danlos type IV as well as provide evidence for a novel genetic defect associated wtih the Ehlers-Danlos phenotype.

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MORPHOLOGICAL ANALYSIS OF AMNIOTIC FLUID CELLS; ITS CONTRIBUTION TO PRENATAL DIAGNOSIS OF SEVERE GENODERMATOSES. Masashi Akiyama, Karen A. Holbrook, Departments. of Biological Structure and Medicine (Dermatology), University of Washington School of Medicine, Seattle, WA.

Several severe genodermatoses cannot yet be diagnosed in utero on a molecular or biochemical basis but can be recognized by the structure of a fetal skin biopsy sample obtained at 18-20 wks estimated gestational age (EGA). Amniotic fluid (AF), which can be sampled earlier (14-16 wks EGA), may contain skin-derived cells that reflect an affected fetus; e.g., bullous congenital ichthyosiform erythroderma and harlequin ichthyosis (HI). To determine whether populations of AF cells reflect genetic conditions, we surveyed the population of AF cells from 36 fetuses at 16-21 wks EGA at risk of junctional epidermolysis bullosa (EB), recessive dystrophic EB, EB simplex, HI, lamellar ichthyosis (LI) and Sjögren-Larson syndrome (SLS). Periderm cells, keratinocytes, cells of unknown epithelial origin, fibroblasts, fibrin clots and urinary cast-like materials were seen in AFs from normal fetuses. A remarkable number of macrophages phagocytizing collagen fibers were found in AFs from all recessive dystrophic and some junctional EB fetuses although a few macrophages were also observed in AFs from some normal fetuses and fetuses with congenital ichthyosis. Clumps of keratinized cells were observed in AF samples from the fetuses affected with HI and LI. These results indicate that the morphological analysis of AF cells can provide important supportive information for the prenatal diagnosis of several severe genodermatoses.

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EFFECT OF SURFACTANT MIXTURES ON IRRITANT DERMATITIS POTENTIAL IN MAN : SODIUM LAUROYL GLUTAMATE AND SODIUM LAURYL SULPHATE. Yoshiaki Kawasaki*1, Cheol Heon Lee*2, Kazutami Sakamoto*1, Howard I. Maibach*2, *1Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki, JAPAN, *2Department of Dermatology, SChool of Medicine, University of California San Francisco, San Francisco, U.S.A.

Acylglutamate is an anionic surfactant synthesized from two natural occuring moeties, glutamic acid and fatty acid. The pH of its aqueous solution is arround 5.5 which is nearly equal to that of normal human skin. Acylglutamate has been used as a main surfactant for various kinds of cleansing products which offers mildness as main concept.

The present study delineates the mildness of sodium lauroyl glutamate (SLG) and assesses its irritating potential in surfactant mixtures with sodium lauryl sulphate (SLS) on human skin (fifteen adult volunteers free of skin disease and with no history of atopic dermatitis) with using visual scoring and transepidermal water loss (TEWL) measurement.

The visual scores and TEWL values of 1% SLG show lower than those of the other test solutions. 1% Surfactant mixture solution (SLG and SLS) showed lower visual scores and TEWL values than 1% SLS solution. Increase of SLG concentration deceased the visual scores and TEWL values.

These findings suggest that SLG is a mild surfactant and can decrease irritation potential of SLS in mixture.

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LAMELLAR BODIES AND STRATUM CORNEUM EXTRACELLULAR COMPARTMENTS ARE ACIDIFIED: IMPLICATIONS FOR BARRIER HOMEOSTASIS. Stephen Grayson, Martin J. Behne, Man Mao-Qiang, Kenneth R. Feingold, Peter M. Elias. Dermatology and Medical Service, VAMC and Departments of Dermatology and Medicine, University of California, San Francisco, California, U.S.A.

The acid hydrolases found in lamellar bodies (LB) and the stratum corneum extracellular (SCE) compartments are activated optimally at an acidic pH. Moreover, recent studies have shown that extracellular processing of LB-derived lipid precursors by at least one acid hydrolase, ß-glucocerebrosidase, is necessary for the formation of SC lamellar bilayers and the formation of a competent permeability barrier. Thus, inhibition of SCE acidification should prevent the normal processing of LB-derived lipids and interfere with permeability barrier homeostasis. We asked whether LB are acidified through the action of vacuolar proton pumps (V-ATPase), and whether the SCE compartments are acidified by the secretion of LB contents and continued action of these pumps. We show here that the lysosomal marker LAMP, and V-ATPase accumulate in the outer epidermis where LB are found. These results suggest that LB may be related to lysosomes and that they are probably acidified immediately before they fuse with the apical plasma membrane and secrete their contents. Whereas the punctate orange fluorescence observed after treatment with acridine orange (AO) is prevented by incubation of upper epidermal sheets with monensin or the specific V-ATPase inhibitor, bafilomycin A1, punctate fluorescence from the pH fluorescent probe, carboxy SNAFL-diacetate, in outer epidermal sheets and frozen sections appears to indicate the presence of neutral or slightly alkaline vesicles but acidic SCE compartments. These results confirm that LB are acidified at a very late stage in terminal differentiation. Finally, recovery of the permeability barrier in mice treated with acetone is significantly faster in an acidic (e.g., pH 5.5) versus a neutral (e.g., pH 7.4) environment. These results suggest that: 1) LB are acidified by V-ATPase late in differentiation; and that 2) SCE acidification is required for permeability barrier homeostasis.

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BARRIER FUNCTION AND STRATUM CORNEUM WATER HOLDING CAPACITY: EFFECT OF SITE AND MENSTRUAL CYCLE. Enzo Berardesca, Fernanda Distante, Gian Piero Vignoli, Giacomo Rabbiosi, Dept. of Dermatology University of Pavia, IRCCS Policlinico S. Matteo, Pavia, Italy

Regional differences in skin physiology occur even in areas of the skin with similar anatomical structure. In this study we have evaluated in vivo barrier function and stratum corneum water holding capacity during the menstrual cycle on the volar forearm (upper and lower) and on the anterior aspect of the thigh using the plastic occlusion stress test (POST). 13 healthy women (age 31±4) with regular menses entered the study. Measurements were taken on the 10th and 25th day of the menstrual cycle. POST was performed by applying a plastic chamber (1.8 cm diameter) on the skin for 24 hrs. At the removal Skin Surface Water Loss (SSWL) was measured using an evaporimeter (EP1-Servomed, Sweden) every 5 min for 30 min. Statistical analysis was performed using one factor ANOVA for repeated measures. Free and bound water compartments of evaporation were also analyzed. Higher hydration and SSWL were detectable at the 25th day of the cycle. However, no significant influence of menstrual cycle was found. Significant differences between the upper and lower volar foream were detected ($P<0.001$). These differences were mainly related to the evaporation of bound water confirming a different barrier function in these sites and supporting the view that adjacent skin sites with equal structure may have different functional behaviour.

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EPIDERMAL CALCIUM GRADIENT AND REGULATION OF BARRIER HOMEOSTASIS. Gopinathan K. Menon, Peter M. Elias, Kenneth R. Feingold. Veterans Administration Medical Center, San Francisco, CA.

Normal epidermis, with an intact permeability barrier, possesses a characteristic Ca^{++} gradient, with low extracellular Ca^{++} in the basal and spinous layers and high extracellular Ca^{++} in the stratum granulosum (SG). Acute disruption of the barrier by solvent treatment or tape stripping results in an immediate loss of the gradient due to passive loss of Ca^{++} through the stratum corneum (SC). The initial phase of barrier repair, which is mediated primarily by lamellar body secretion from SG cells, proceeds in a low extracellular Ca^{++} milieu. As barrier repair progresses, the Ca^{++} gradient normalizes. Artificial restoration of the high extracellular Ca^{++} milieu immediately following barrier disruption inhibits lamellar body secretion and barrier repair.

In both humans and mice, chronic barrier dysfunction (e.g., as occurs in psoriasis, essential fatty acid deficiency, and with lovastatin treatment) results in loss of the Ca^{++} gradient due to increased Ca^{++} content in the lower epidermis. Additionally, Ca^{++} appears in the SC, a site usually devoid of Ca^{++}. The increase in epidermal Ca^{++} in chronic barrier dysfunction is due to enhanced and continuous flux of water/body fluids carrying ions (Ca^{++} and others) through the epidermis. Occlusion of the skin with a water-vapor impermeable membrane decreases both fluid flux and restores the Ca^{++} gradient towards normal. Together, these results suggest that permeability barrier integrity maintains the epidermal Ca^{++} gradient.

More recently, we have studied the effects of ionic changes, independent of barrier disruption and increased TEWL, on lamellar body secretion. We altered the extracellular Ca^{++} gradient with sonophoresis, which does not perturb the barrier. Sonophoresis of Ca^{++}-free sucrose solutions resulted in the translocation of the Ca^{++} reservoir into the dermis, resulting in depletion of extracellular Ca^{++} in the SG. This change was accompanied by immediate secretion of lamellar bodies. In contrast, sonophoresis of Ca^{++}-containing solutions resulted in increased Ca^{++} at all levels of the epidermis, without stimulating LB secretion by SG cells. These findings demonstrate that changes in the extracellular Ca^{++} content surrounding SG cells is an important signal for lamellar body secretion, the initial event in barrier repair following acute perturbations.

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LOCALIZATION OF EPIDERMAL CYTOKINE RESPONSES TO BARRIER PERTURBATION. Jui-Chen Tsai, Peter M. Elias, LaDonna C. Wood, Kenneth R. Feingold. Dermatology and Medicine Services, Veterans Affairs Medical Center, San Francisco, California, U.S.A.

Epidermal barrier perturbations elicit a carefully orchestrated repair response, including lamellar body (LB) secretion and enhanced epidermal lipid and DNA synthesis, leading to barrier repair. Since epidermal cytokine expression increases with barrier disruption, these substances could signal the repair sequence. Thus, we localized TNFα and IL-1α by immunohistochemistry within murine epidermis before and after barrier perturbation with acetone or tape stripping, and in essential fatty acid deficiency (EFAD). Whereas TNFα immunostaining was low in untreated epidermis, it increased throughout the nucleated layers immediately after either acetone or tape stripping, normalizing between 4 and 6 hrs. Moreover, the increase in TNFα content following barrier perturbation was also shown by Western immunoblotting. In contrast, IL-1α staining was more intense than TNFα in control epidermis, and concentrated in the outer epidermis. After acute perturbations, IL-1α staining increased slowly, remaining elevated at least 4-6 hrs, and became redistributed in the cytosol of all the nucleated layers. In EFAD epidermis, TNFα staining was increased slightly in all of the nucleated cell layers, with the greatest concentration in the stratum corneum. The relationship of the cytokine response to barrier function was shown further in occlusion studies, where the expected increased in TNFα 2 hrs after acetone treatment was aborted by prior application of a vapor-impermeable membrane. These studies show: a) both differences and similarities in the baseline distribution of TNFα and IL-1α in untreated murine epidermis, and b) further differences in localization with barrier perturbation consistent with a role for these cytokine sin regulating the repair response that follows barrier perturbation.

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VINCULIN IN TWO DIFFERENT METASTATIC MOUSE MELANOMA CELL LINES - A QUALITATIVE STUDY USING CONFOCAL LASER SCANNING MICROSCOPY. **R Fink-Puches, J Smolle, R Hofmann-Wellenhof, C Helige, H Kerl. Department of Dermatology, University of Graz, Graz, Austria**

Vinculin containing focal contacts are the points at which cells make their closest contact with the extracellular matrix and provide the primary stabilizing force for cell attachment. Another major fraction of cellular vinculin is a diffusible cytoplasmic pool, which maintains a dynamic equilibrium with the membrane bound fraction. The abilities of malignant cells to bind and migrate on extracellular matrices are important steps in invasion and metastasis associated with tumor progression. Malignant transformation results in loss of cell polarity and diminished cell-substrate adhesion. Altered expression of vinculin may play a role in the capacity of tumor cells to invade tissues and metastasize.

In the present study the amount and distribution of vinculin in two mouse melanoma cell lines (K1735-cl16: low metastatic clone; K1735-M2: high metastatic clone) cultured on glass was investigated using an indirect immunofluorescence technique and confocal laser scanning microscopy (CLSM). Both melanoma cell sublines revealed a diffuse staining of the cytoplasm, as well as strongly stained foci, indicating the vinculin containing contacts. The low metastatic cell line showed a dense distribution of vinculin-rich plaques at the margin of the cells and only few plaques on the whole ventral surface. In contrast, the high metastatic cell line K1735-M2 revealed the vinculin-containing contacts throughout the entire ventral surface of the cells irregularly disseminated at the plasma membrane.

We conclude, that CLSM helps us to detect the vinculin antibody by recording optical sections through the cells. The use of confocal mode ensures, that all vinculin-detected plaques are on the ventral surface of the cells. Different metastatic mouse melanoma cell sublines show an altered distribution of vinculin-rich plaques on the ventral surface of the cells cultured on glass, which might indicate different intractions of the cells with the substratum.

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CONFOCAL LASER SCANNING MICROSCOPIC EXAMINATION OF THE CUTANEOUS INNERVATION OF NORMAL HUMAN AND ALOPECIA AREATA (AA) SCALP ANAGEN HAIR FOLLICLES. Maria Hordinsky, Michelle Bussmann, *Gwen Crabb, Sunny Lewis, *William Kennedy. Departments of Dermatology and *Neurology, University of Minnesota, Mpls. MN., USA.

The innervation of scalp hairs has been described as originating from a plexus in the deep dermis with nerve fibers ascending parallel to the follicle forming terminal axons above the bulge and below the entrance of the sebaceous glands, descending along the follicle and wrapping themselves around the bulb. Cutaneous innnervation of scalp epidermis has not been reported.

Some individuals affected with AA describe itching, tingling, formication, or slight local pain with tension on their hair. Abnormal EEG tracings and a decreased number of active sweat glands suggest the possibility of a general neurologic defect in AA patients.

We examined the innervation of scalp biopsy specimens from two males (1 alopecia totalis (AT), 1 AA), 3 females with extensive AA and age- and sex- matched controls. The innervation of biopsy samples was examined using antibody to PGP 9.5 and immunohistochemical techniques to stain cutaneous nerves which were then visualized using an epifluorescence microscope and a confocal scanning laser microscope. Our analysis focused on the innervation of the epidermis, middle third of the hair follicle (the bulge region), and hair bulb region.

In all normals surveyed, extensive innervation was seen around the hair bulb and bulge region, but the number of axons innervating these structures varied widely. In two of the matched pairs, innervation of the middle third of the hair follicle and hair bulb was increased in the AA samples. Nerve fibers were found in the epidermis of both patients and controls.

We have demonstrated that nerve fibers are present in the epidermis of normal and AA scalp skin. We assume from their location that these are sensory nerves. We have also identified differences in cutaneous innervation of the hair follicle between patients and controls. The presence of these differences and the demonstration of epidermal nerves may be important in explaining the neurologic symptoms some AA patients experience.

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CHANGES IN OXIDATION STATE AND ANTIOXIDANT ACTIVITY OF RAT SKIN HOMOGENATES WITH AGE. YP Kim*, SY Yang, SH Sohn, BW Ahn, MW Lee, Department of Dermatology*, and Biochemistry, Chonnam University Medical School, Kwangju, Korea

The oxidative damage of tissues by reactive oxygen species has been considered one of the cause of aging process. The effects of aging on lipid peroxidation, protein oxidation, and antioxidant activity of the skin homogenates were investigated in male Sprague-Dawley rats at 3 and 12 months of age. Lipid peroxidation (nmol MDA/mg protein) of skin homogenates showed decreasing tendency, but protein oxidation (nmol carbonyl groups/mg protein) showed increasing tendency during aging. Superoxide dismutase activity (U/min/mg protein) decreased to 60% at 12 month-old rats and catalase (k/mg protein) showed no change with age, but glutathion peroxidase activity (nmol NADPH/min/mg protein) increased to 127% at 12 month-old rats. Alpha-Tocopherol, a major antioxidant in biomembranes, and reduced glutathione (GSH) did not show statistically significant change, but the GSH/GSSG ratio was 1.5 times higher at 12 month-old rats than 3 month-old rats.

These results may indicate that age-related increase in protein oxidation and decrease in lipid peroxidation might be due to the decrease in superoxide dismutase activity that can protect proteins and to increase in glutathion peroxidase activity and GSH/GSSG ratio that can protect lipids from oxidative attack, respectively.

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TRICHOTHIODYSTROPHY (TTD) HAIR PRODUCTION BY TTD-SCALP GRAFTS MAINTAINED ONTO *NUDE* MICE: BIOCHEMICAL STUDY. B. de Brouwer (1), K.H. Phan (2), J. Föhles (2), D. J.J. Van Neste (1), (1) Skin Study Center, Skinterface, Tournai (Belgium) and (2) Deutsches Wollforschungsinstitut and der TH Aachen, Aachen (Germany)

Trichothiodystrophy (TTD) is a rare hair defect characterized by low levels of abnormal high sulfur proteins (HSP). Such qualitative and quantitative changes of HSP are associated with extreme brittleness of the hair shaft and the abnormal structure of the cortex and hair cuticle.

In this study we show that the abnormal amino acid composition of the lanugo hairs collected from the scalp of a foetus with TTD (donor) was also expressed in thin terminal hairs produced by the donor scalp follicles grafted onto *nude* mice. The latter showed severe cuticular damage and zones without cuticular cells. The amino acid analysis of lanugo hairs and hair shafts produced by grafted scalp specimens was consistent with findings published in the literature: severe decrease of cys (<50%) and moderate decrease of thr and pro (<80%) with an increase of lys-asp-ala-leu-phe (>50%) and ile (>20%). The characteristic biochemical profile indicates a decrease of HSP and a relative increase of keratins. Hence, TTD gene expression and regulation in the human follicle appears to be independent of systemic host related factors. It is the first time that continuous TTD hair production is maintained under laboratory conditions for a 6 months period.

The present experimental evidence supports conclusions gained from a previous assay with TTD-variant scalp grafts, i.e. that the *nude* mouse bearing human scalp specimens may serve as a clinically relevant laboratory model for evaluating regulation of abnormal gene expression in the hair follicle under well controlled experimental conditions.

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CONSERVATION OF KERATIN INTERMEDIATE FILAMENT STRUCTURE. L. N. Marekov, [1]D. A. D. Parry and P. M. Steinert, NIAMS, NIH, Bethesda MD, USA and [1]Massey University, Palmerston North, New Zealand

During differentiation of the epidermis keratin intermediate filaments (KIF) composed of the K5/K14 pair of chains are replaced by K1/K10 KIF. The present work was undertaken to explore how this may occur by analyzing nearest neighbor alignments of molecules. We introduced lysine-lysine crosslinks in KIF or reconstructed subfilamentous oligomers of K5 and K14 or K1 and K10. The crosslinked proteins were digested, the peptides were recovered and the position of the crosslinks was determined. In almost all cases the same crosslinks were found in KIF or in the subfilamentous forms. Equations from the most frequent crosslinks were derived and used to calculate, by the least squares fitting method, the linear dimensions of the nonhelical linkers and the axial alignments of the rod domain segments. Three major modes of antiparallel alignment of two molecules were found for both types of KIF. In the first (A_{12}) they are almost in register, in the second (A_{22}) the 2B segments are aligned and in the third (A_{11}) the 1B segments are aligned. Since the axial length of one molecule is greater than the repeat length between two molecules, it indicates that similarly directed molecules overlap in a "head to tail" manner by about 1 nm. Furthermore most disease-causing mutations in keratins are due to aminoacid changes where neighboring molecules overlap.

Thus, our new data suggest that exchange of the K5/K14 molecules in a preexisting filament network by K1/K10 molecules can occur simply because both K5/K14 KIF and K1/K10 KIF have identical axial parameters.